

(12) **United States Patent**
Williams et al.

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(54) **MICROFLUIDIC CARTRIDGE FOR PROCESSING AND DETECTING NUCLEIC ACIDS**

(58) **Field of Classification Search**
None
See application file for complete search history.

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Michael T. Kusner, Ann Arbor, MI (US)

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(73) Assignee: **NeuMoDx Molecular, Inc.**, Ann Arbor, MI (US)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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This patent is subject to a terminal disclaimer.

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(21) Appl. No.: **14/755,821**

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Primary Examiner — Jennifer Wecker

(65) **Prior Publication Data**

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(74) *Attorney, Agent, or Firm* — Jeffrey Schox; Ivan Wong

Related U.S. Application Data

(63) Continuation of application No. 13/766,009, filed on Feb. 13, 2013, now Pat. No. 9,101,930.

(60) Provisional application No. 61/598,240, filed on Feb. 13, 2012, provisional application No. 61/667,606, filed on Jul. 3, 2012.

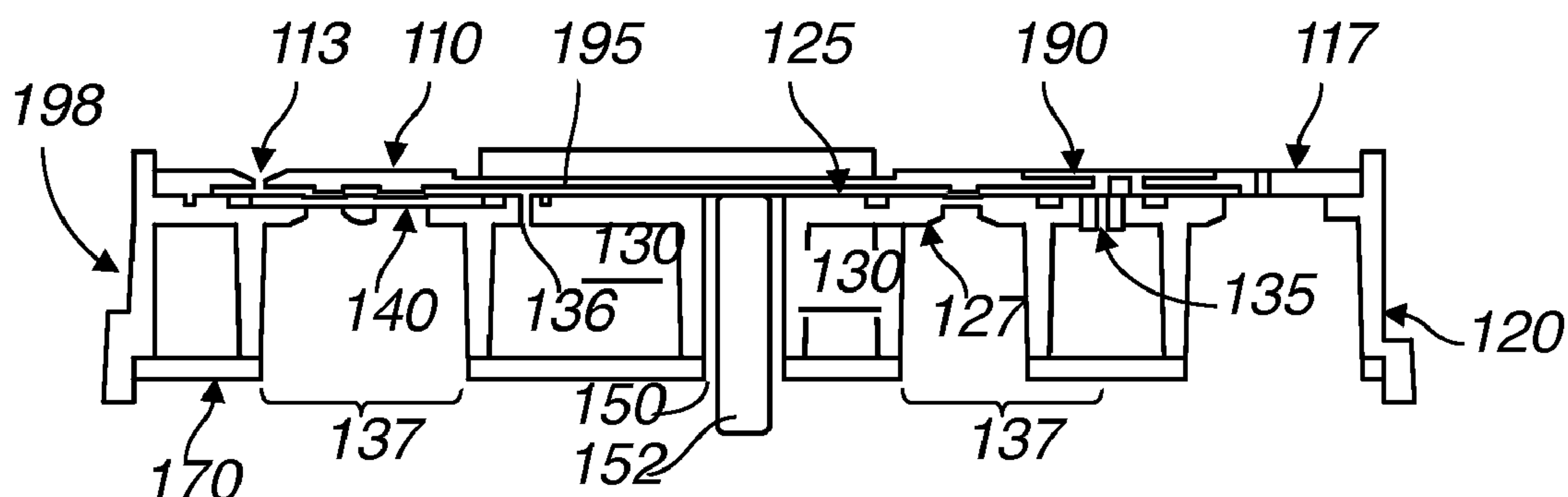
(57) **ABSTRACT**

(51) **Int. Cl.**
B01L 3/00 (2006.01)
C12Q 1/68 (2006.01)
(Continued)

A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region.

(52) **U.S. Cl.**
CPC **B01L 3/502** (2013.01); **B01L 3/5027** (2013.01); **B01L 3/502707** (2013.01);
(Continued)

18 Claims, 18 Drawing Sheets



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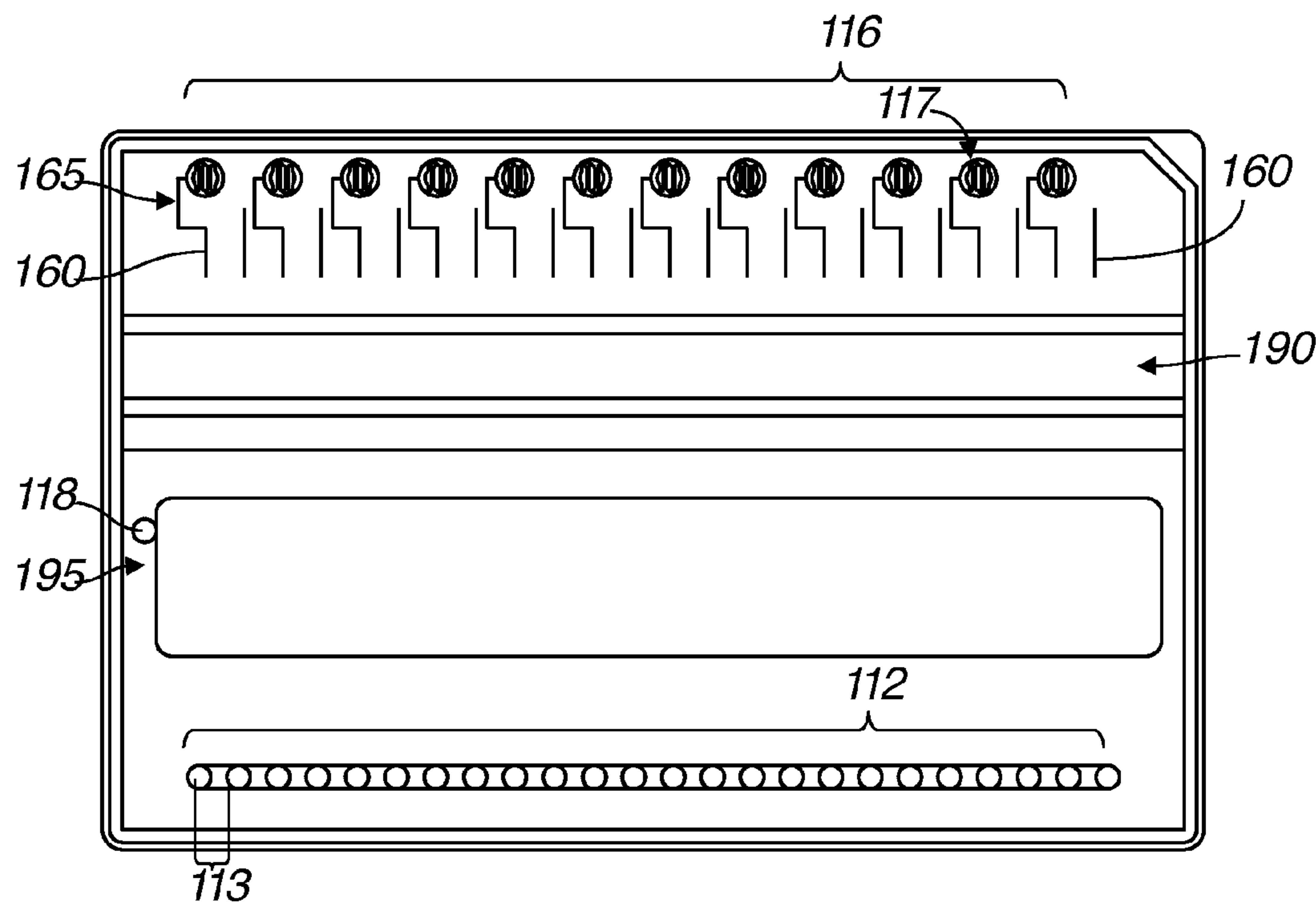


FIG. 1A

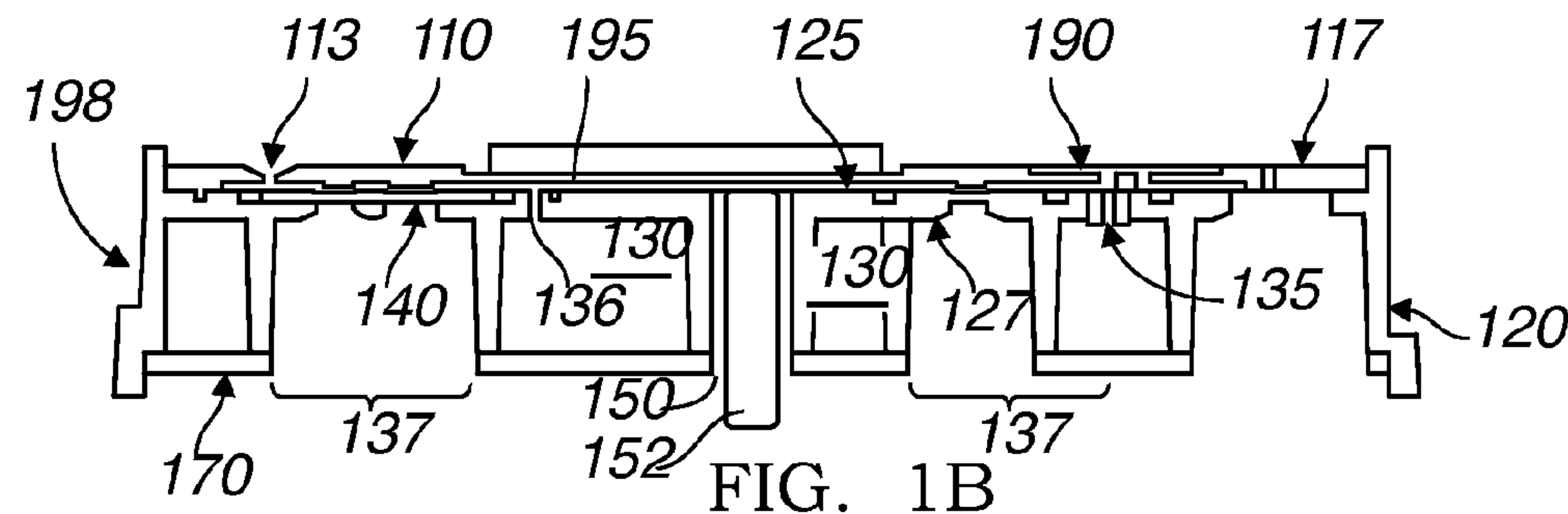


FIG. 1B

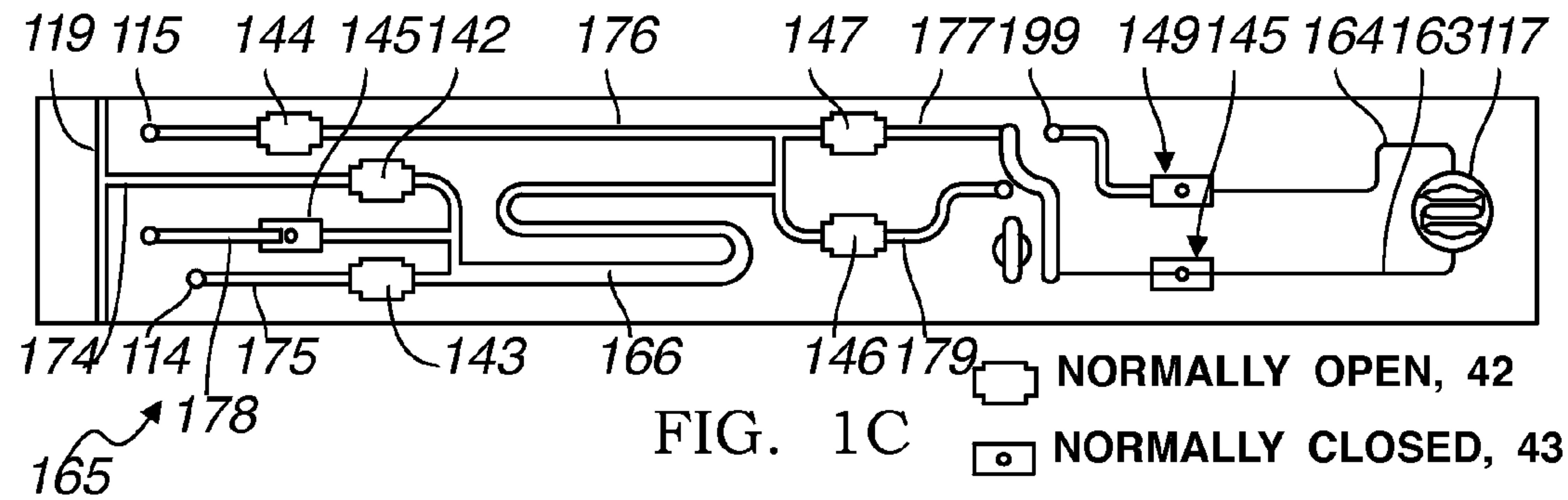


FIG. 1C

NORMALLY OPEN, 42
NORMALLY CLOSED, 43

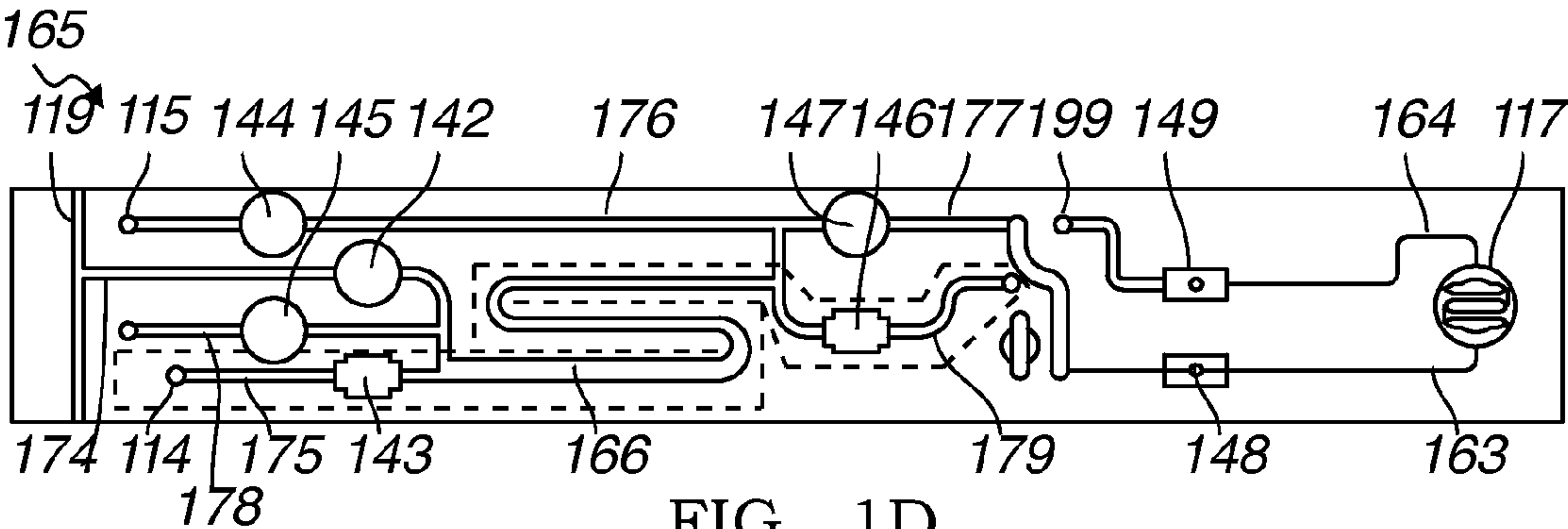


FIG. 1D

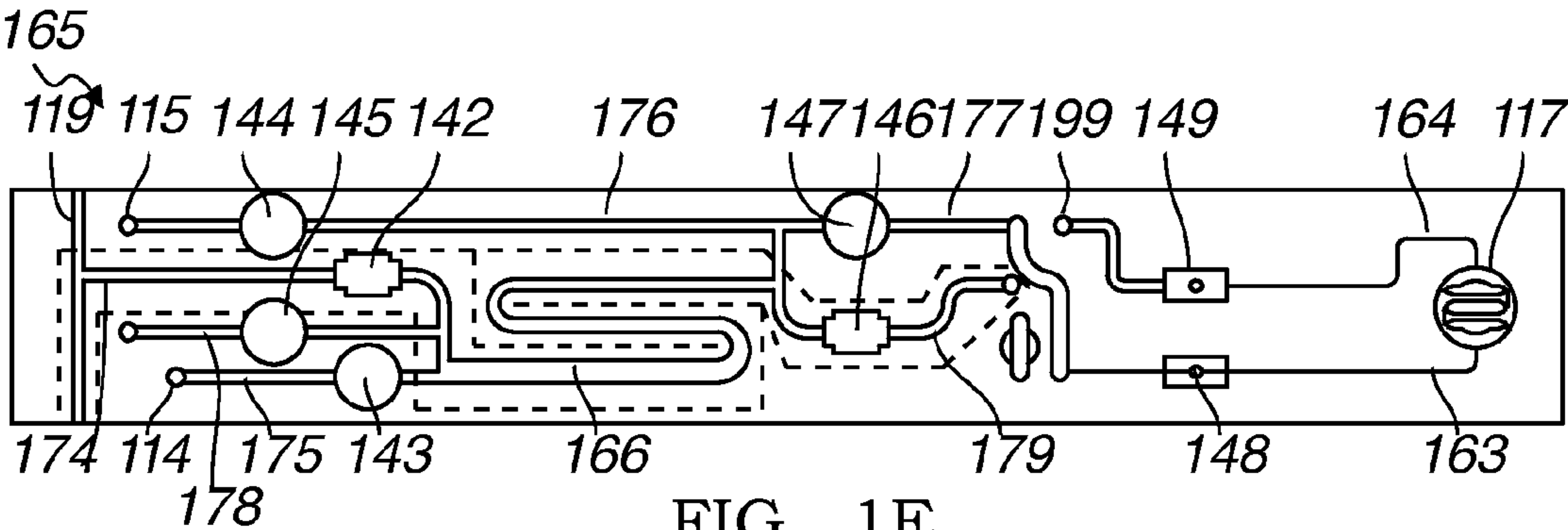


FIG. 1E

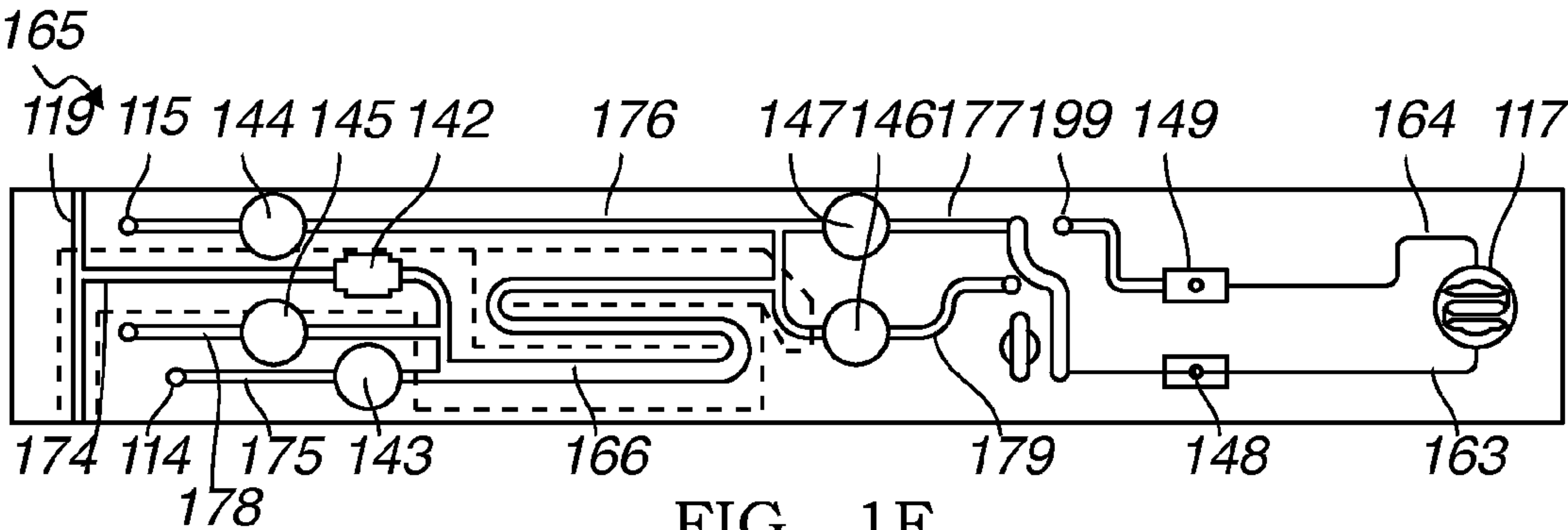


FIG. 1F

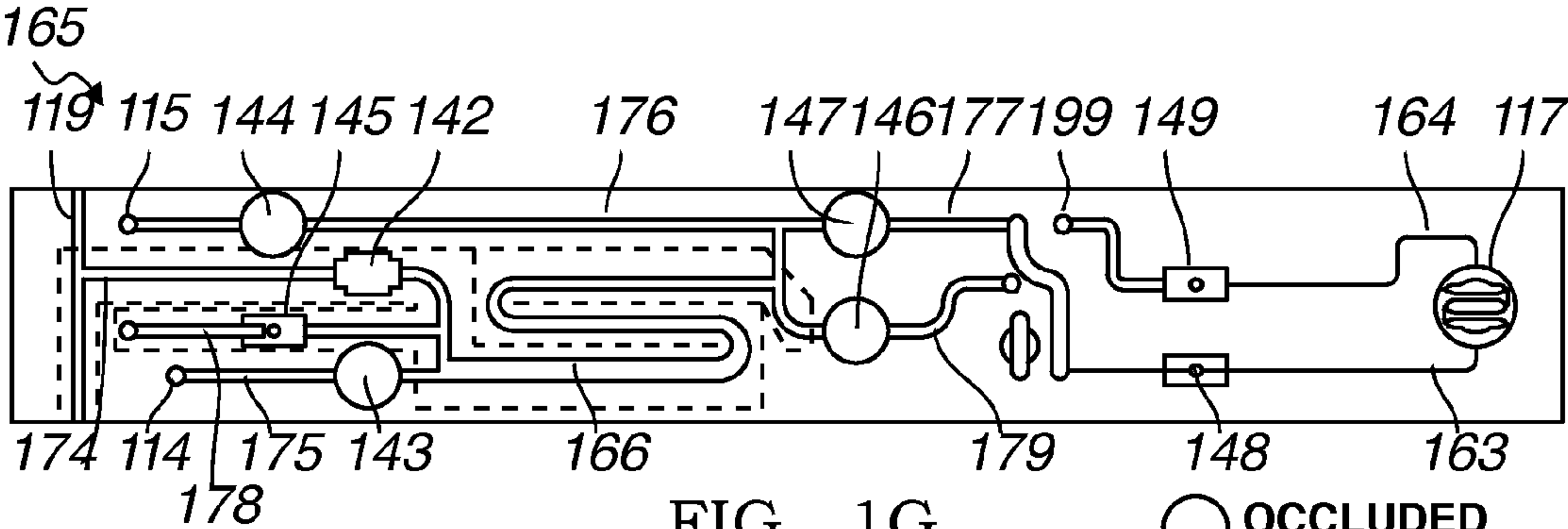


FIG. 1G

○ OCCLUDED

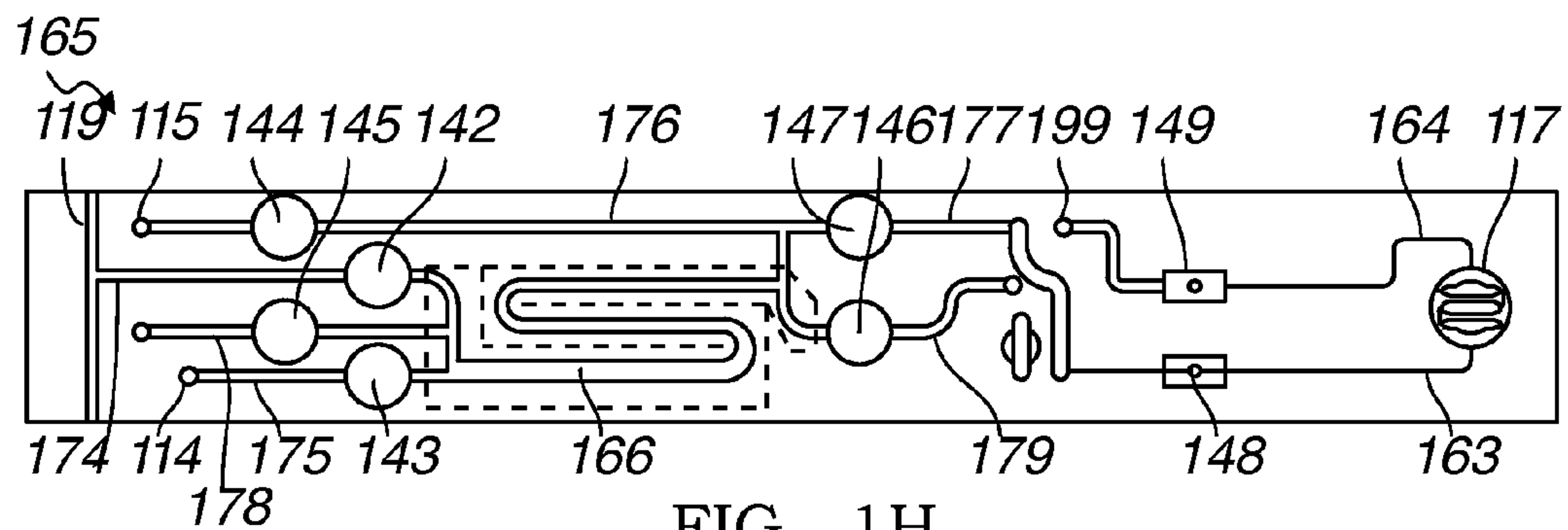


FIG. 1H

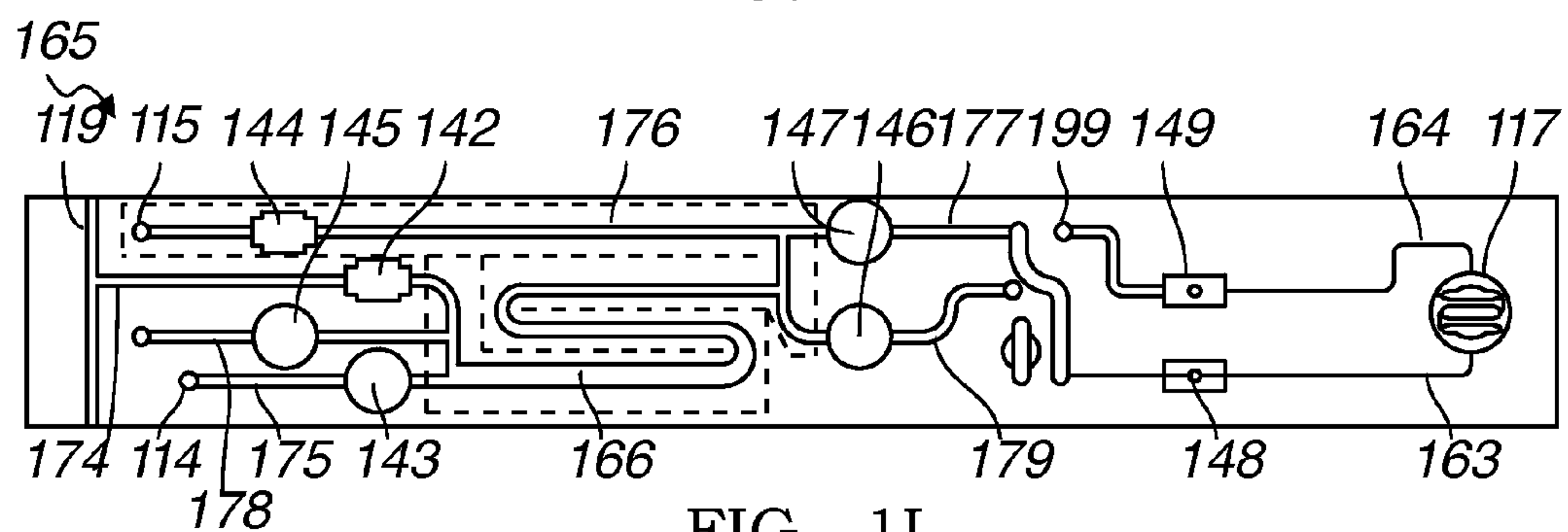


FIG. 1I

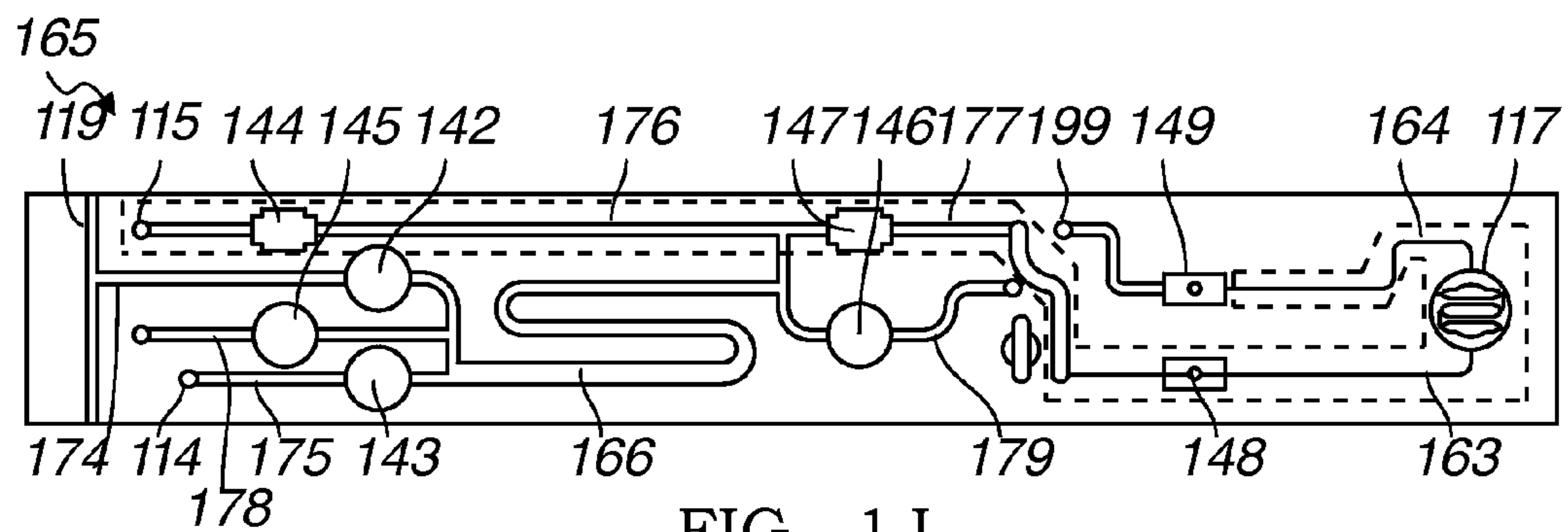


FIG. 1J

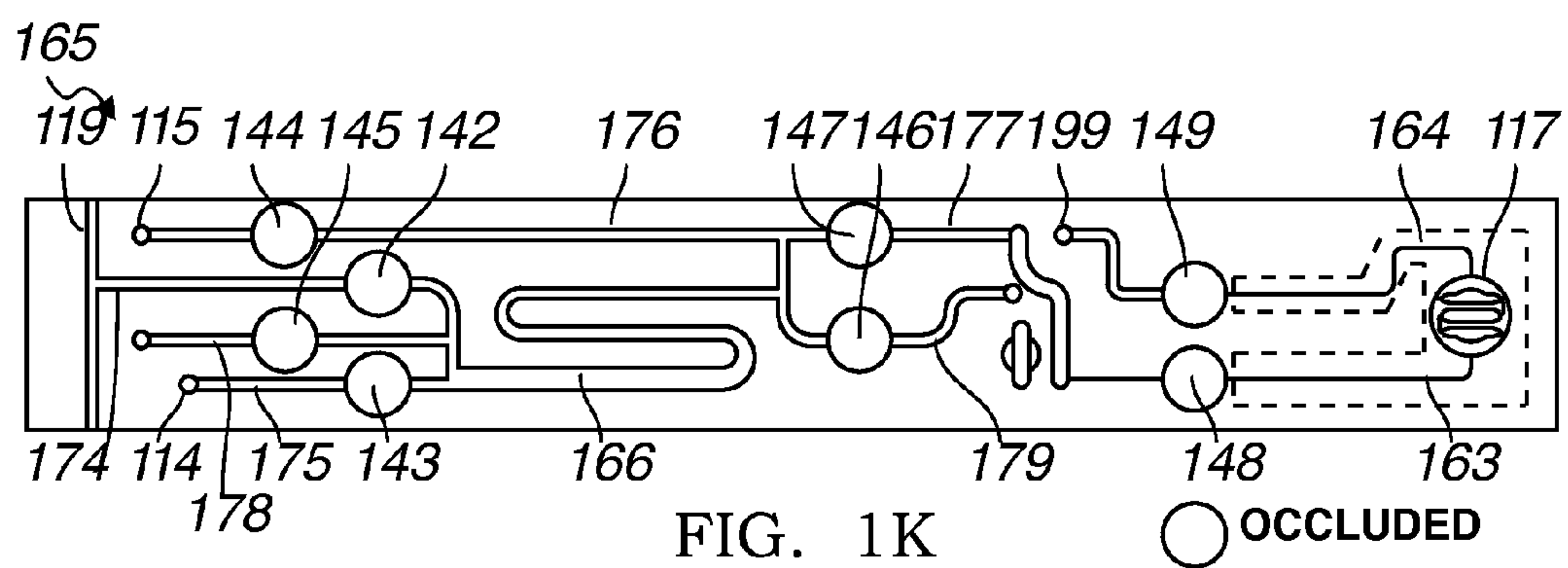


FIG. 1K

OCCLUDED

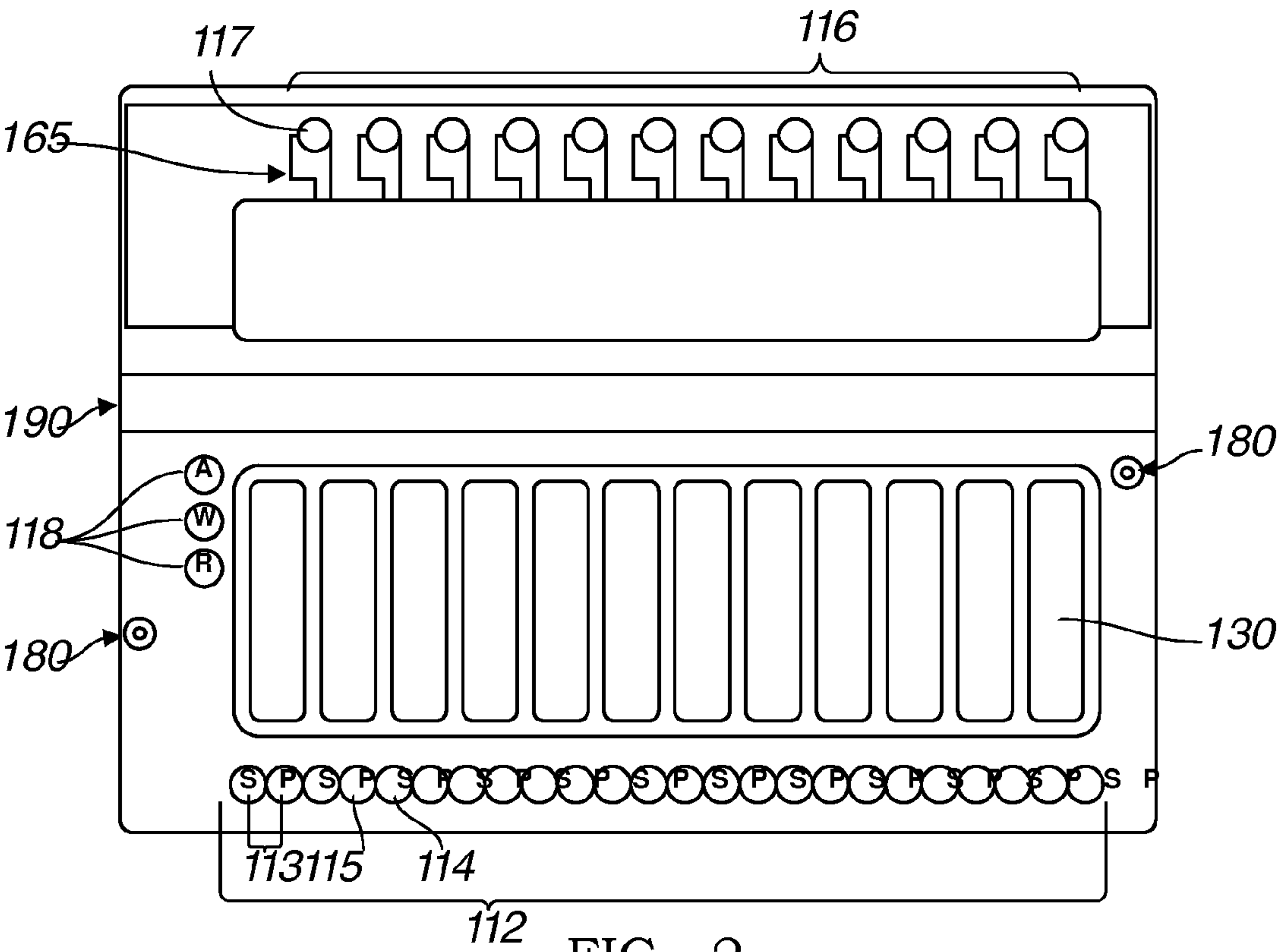


FIG. 2

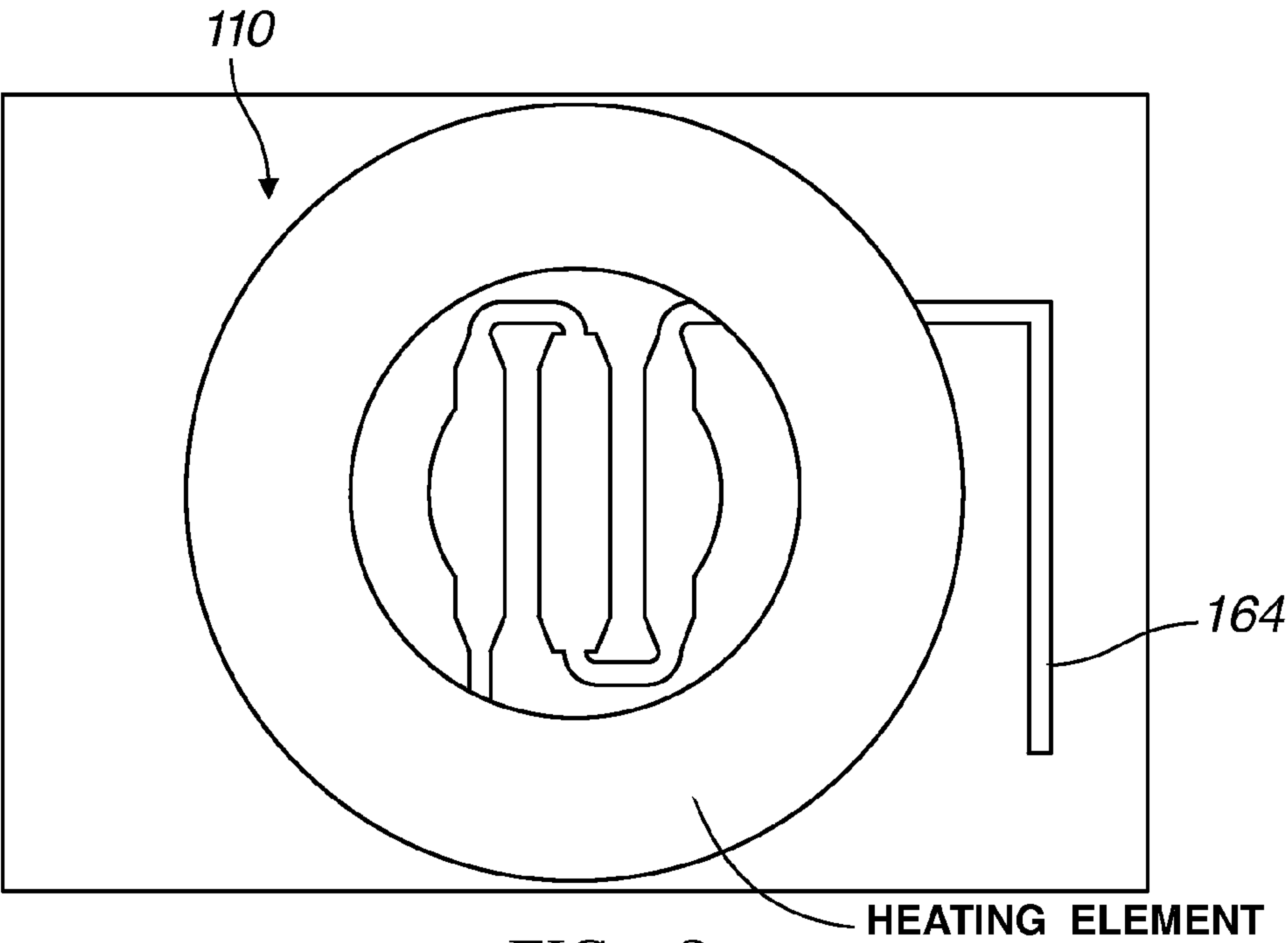


FIG. 3

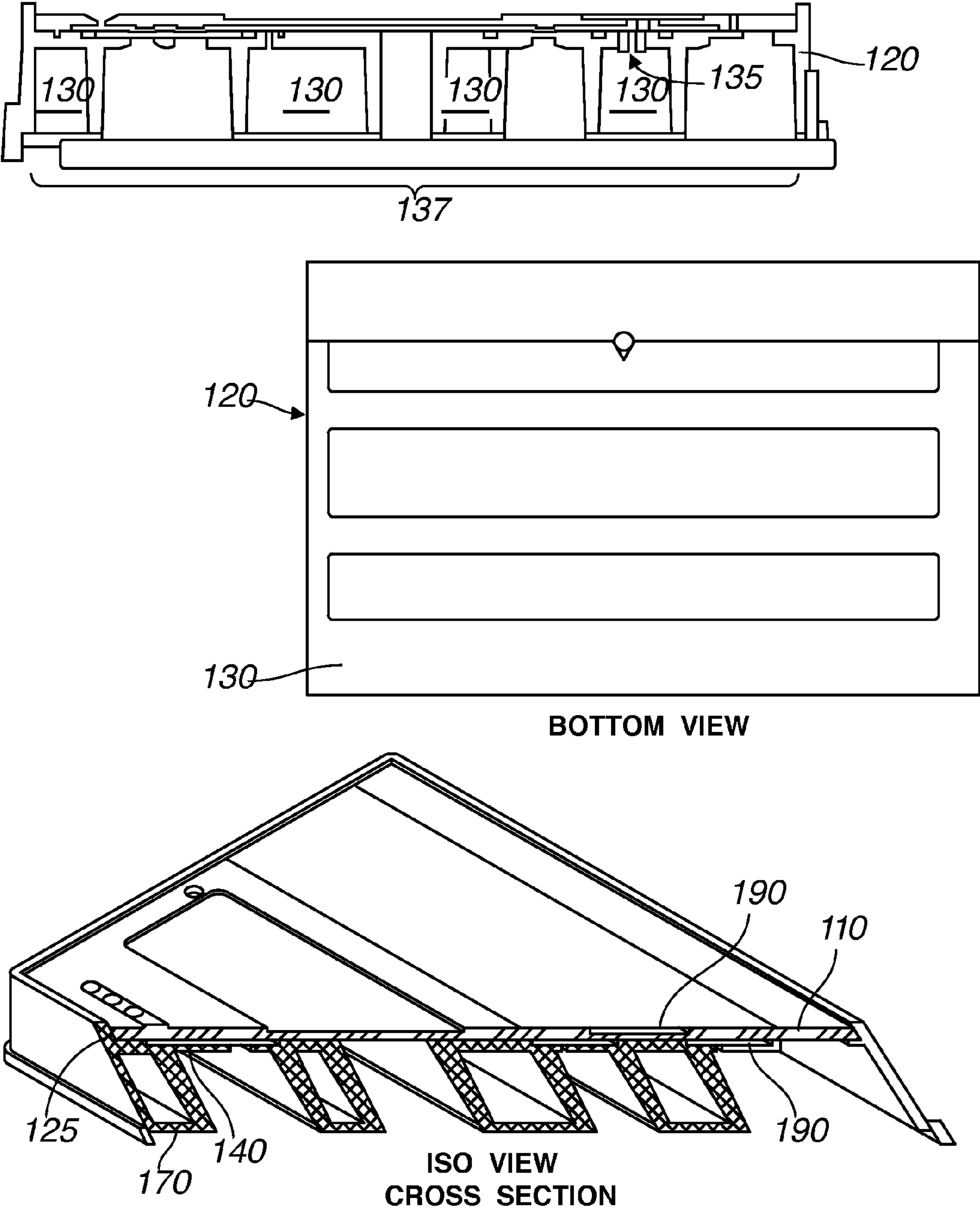


FIG. 4

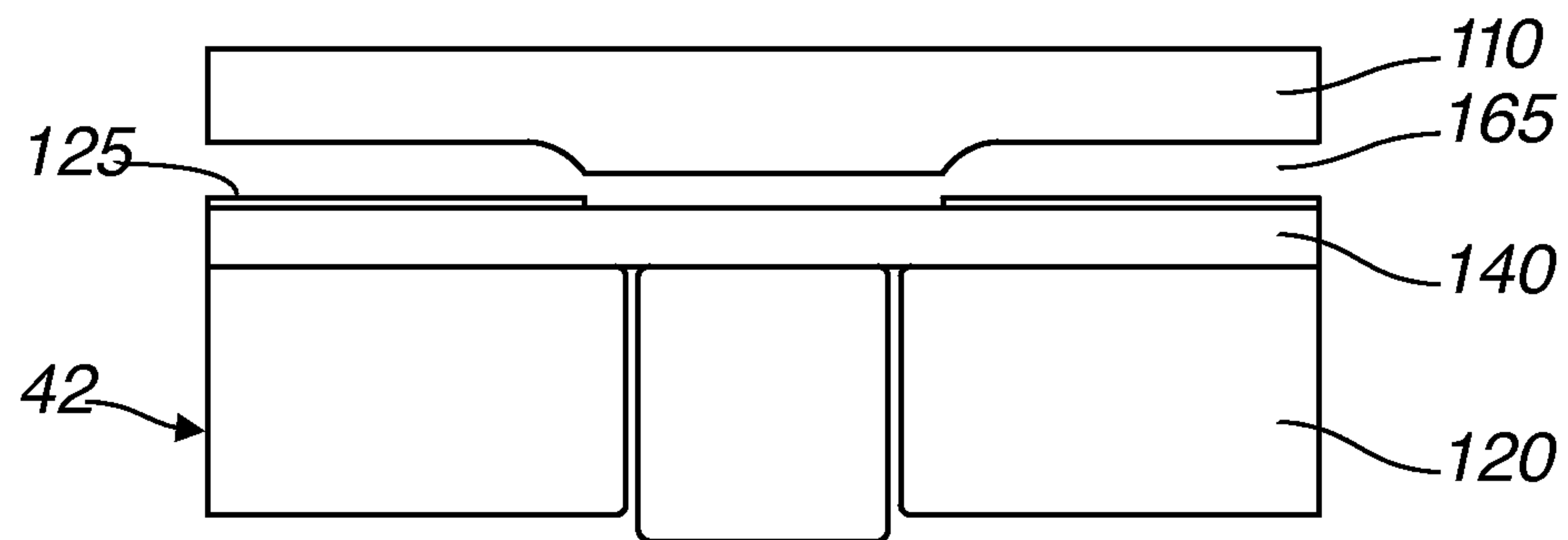


FIG. 5A

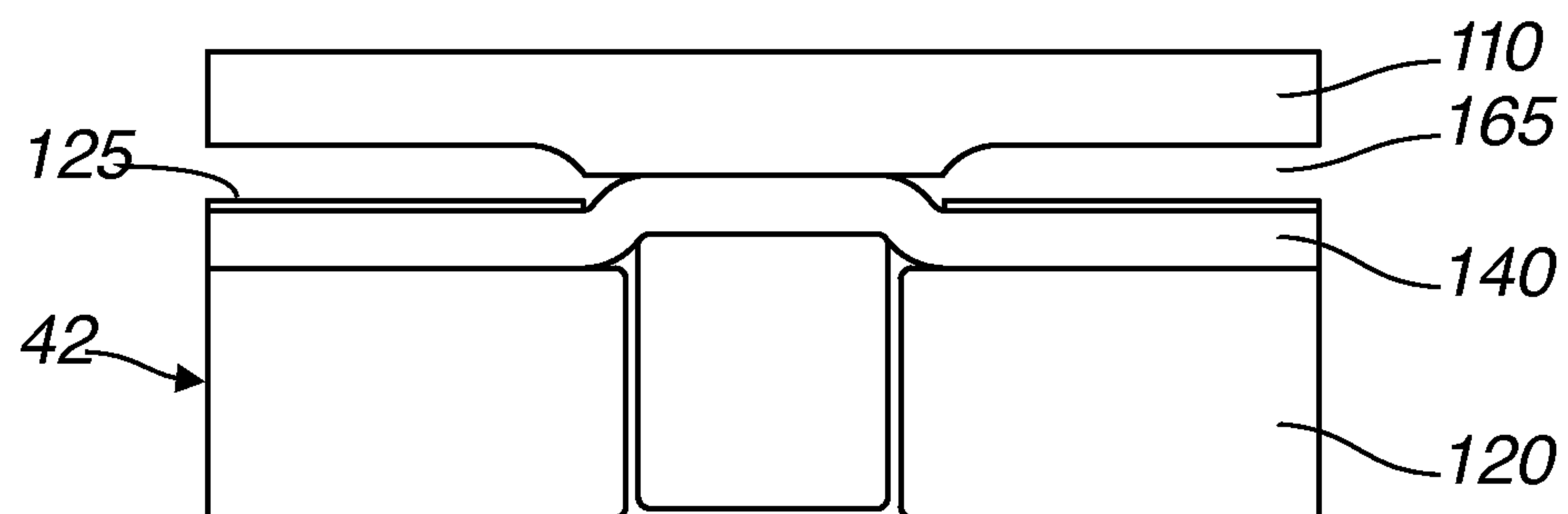


FIG. 5B

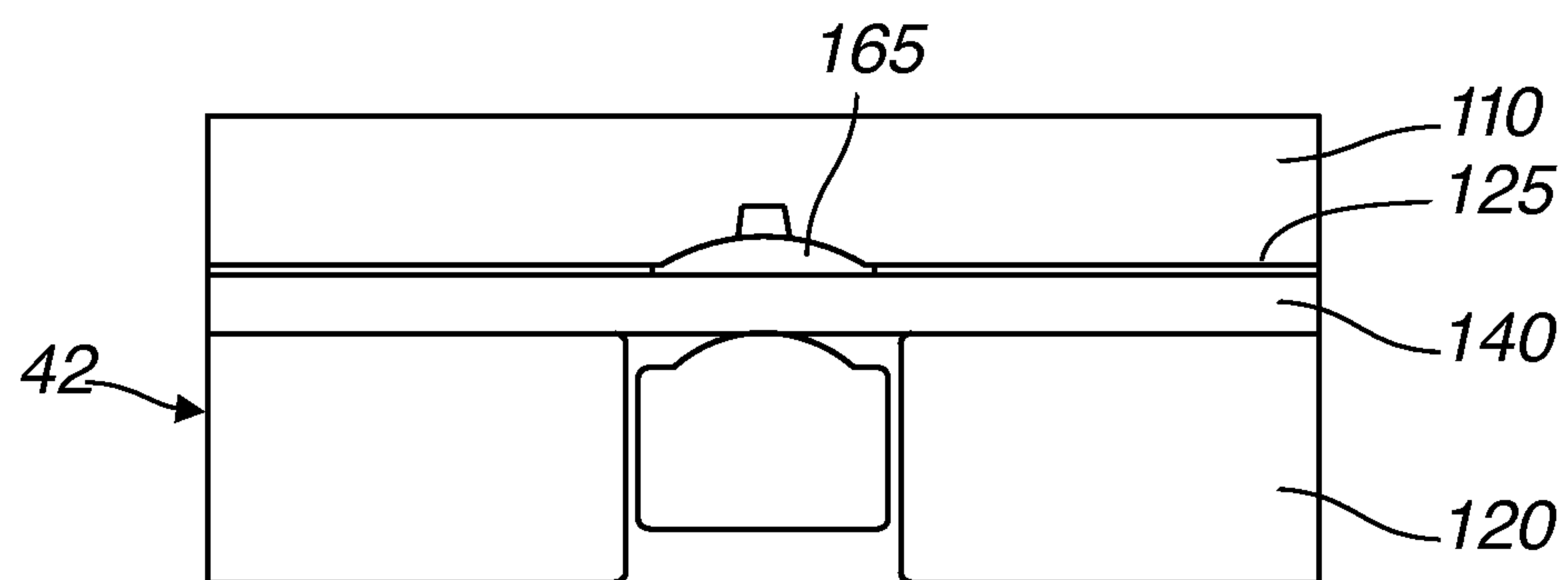


FIG. 5C

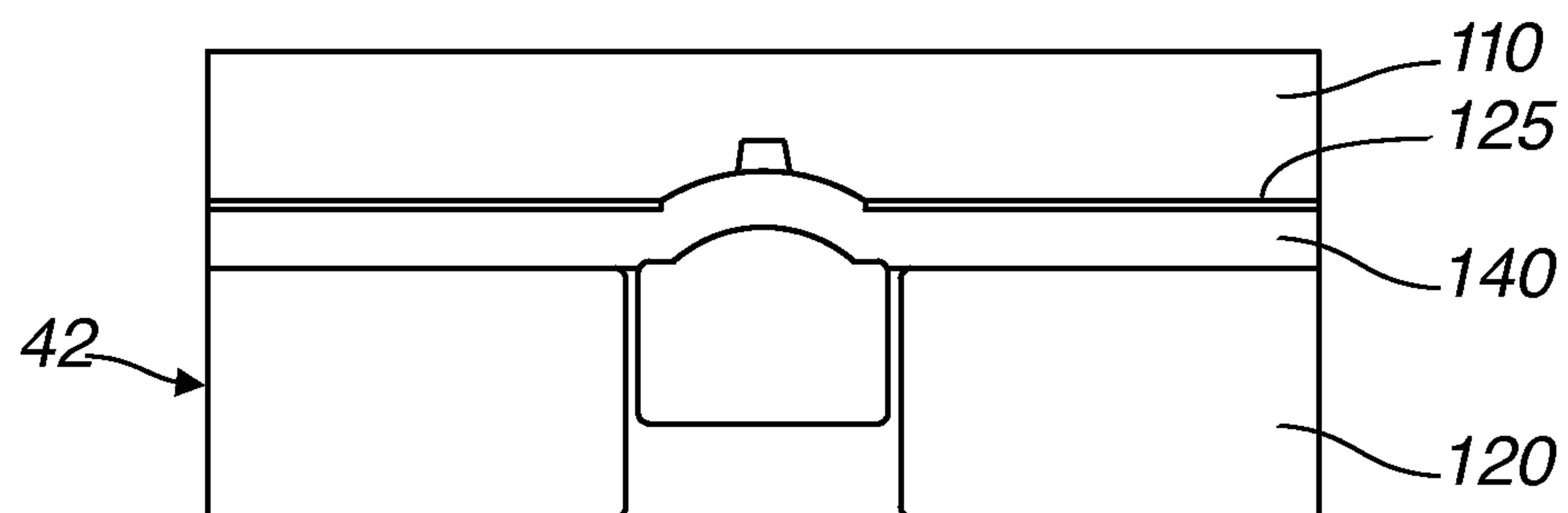


FIG. 5D

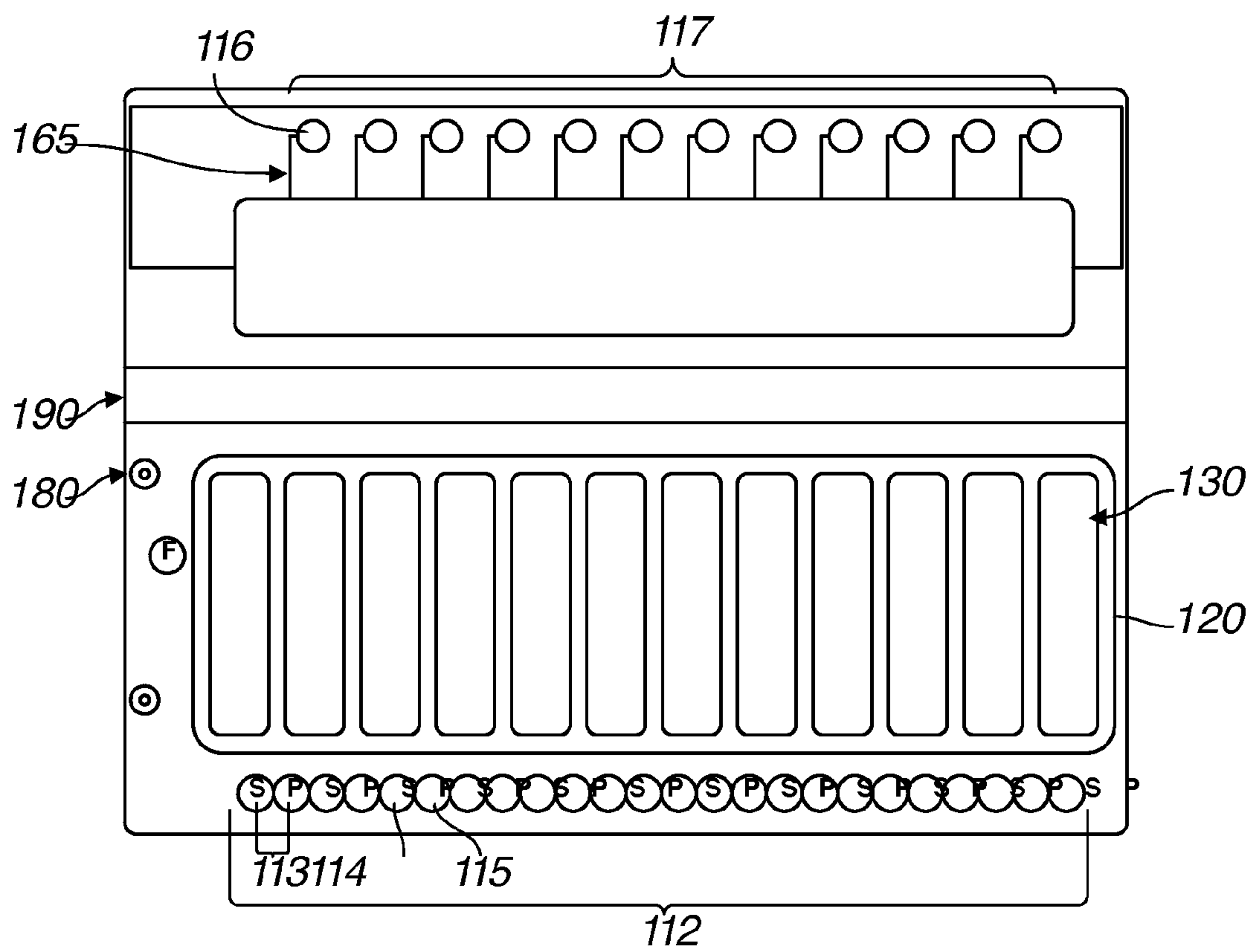


FIG. 6A

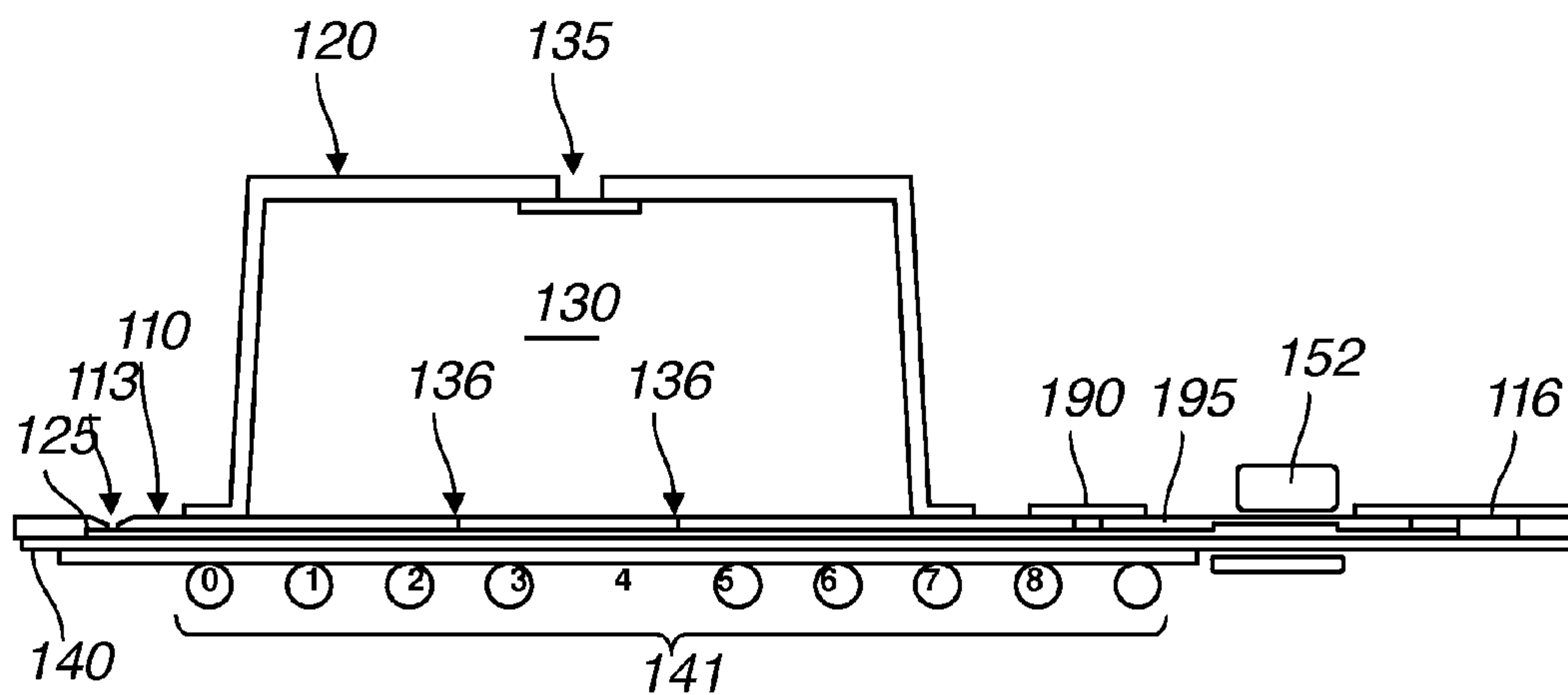


FIG. 6B

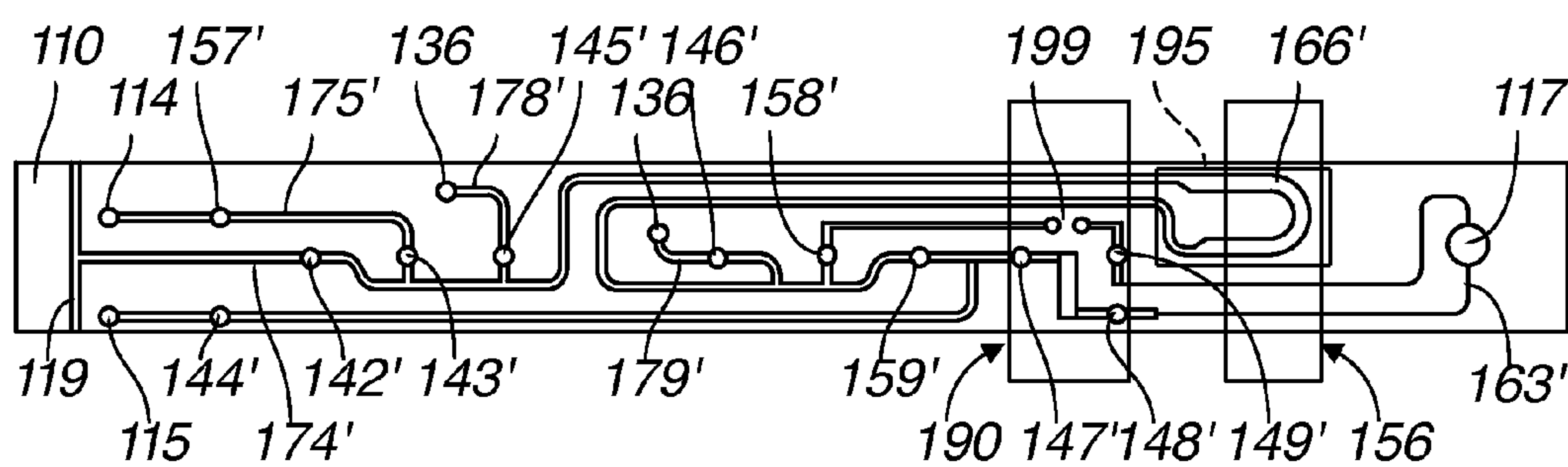


FIG. 6C

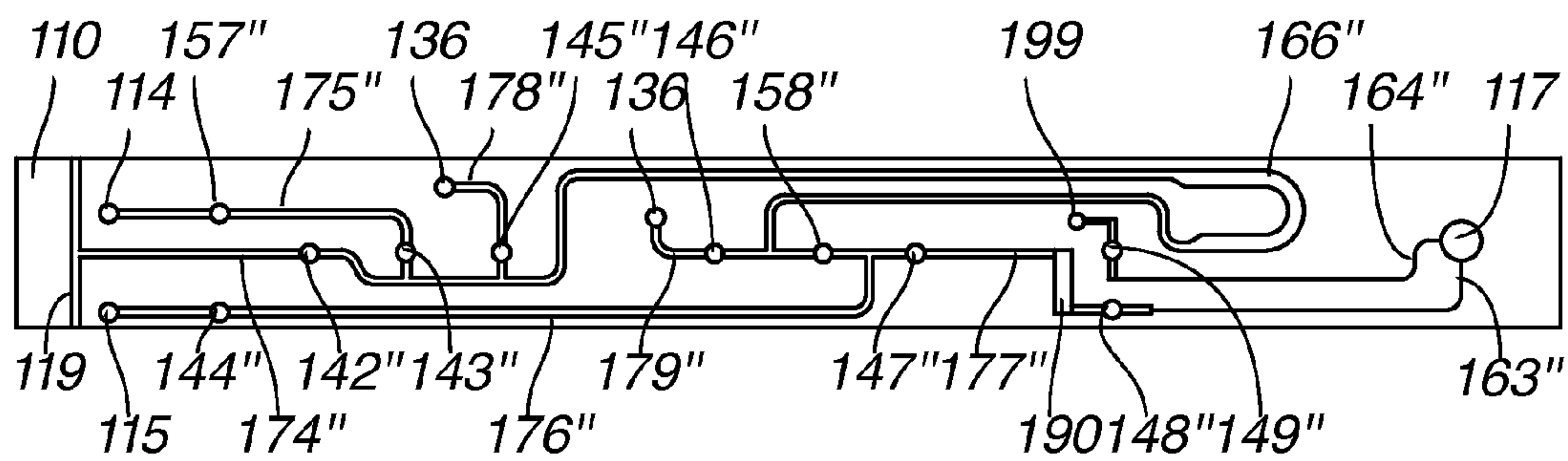


FIG. 7

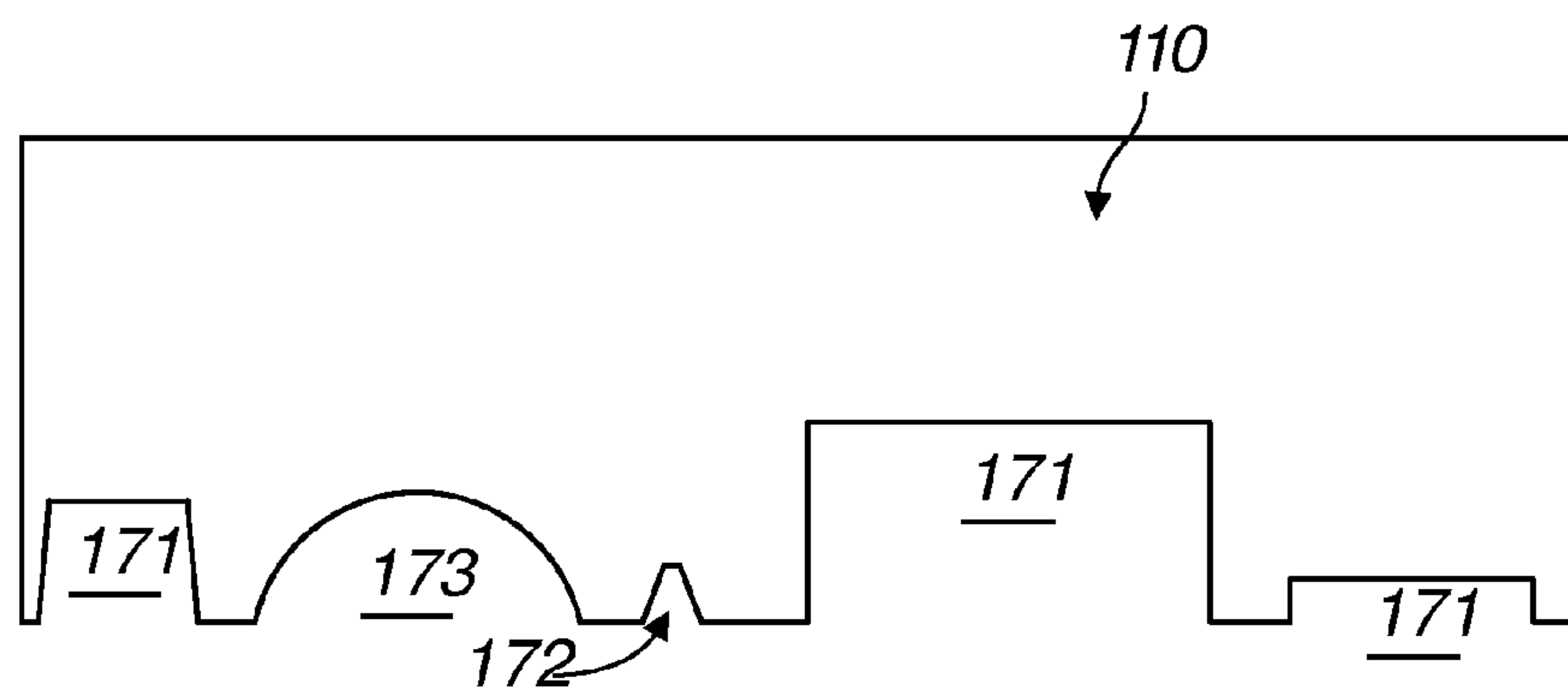


FIG. 8A

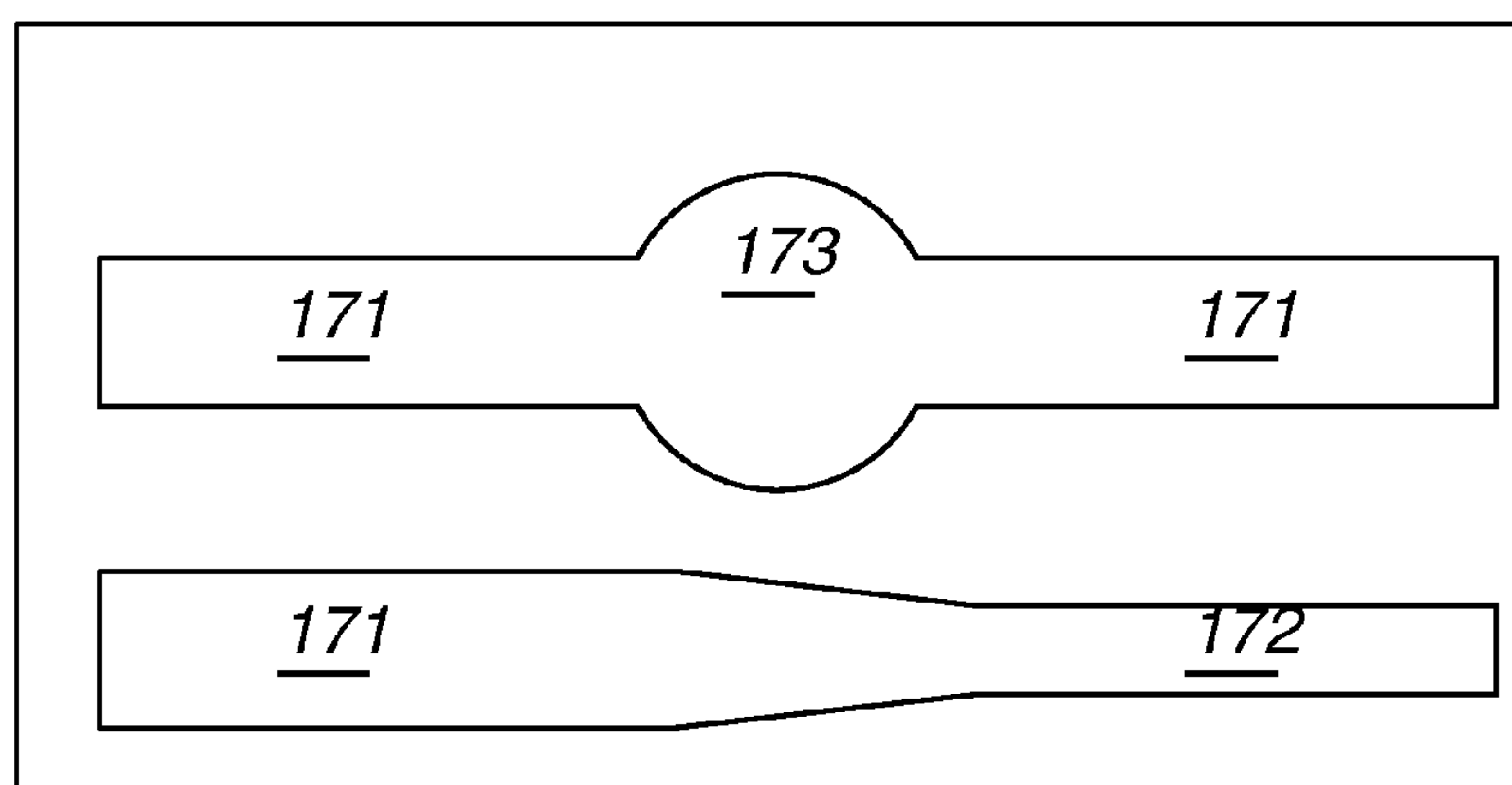


FIG. 8B

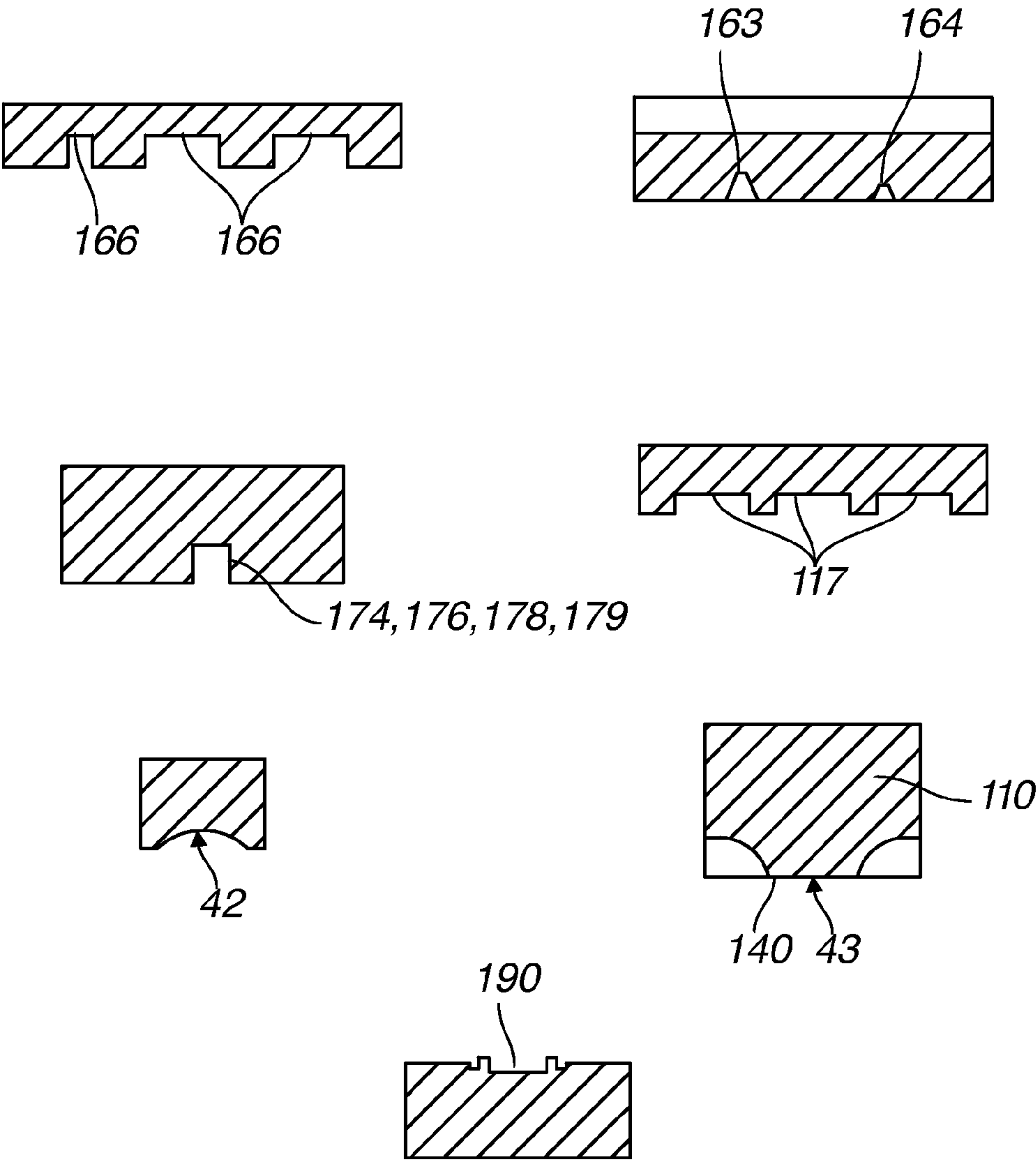
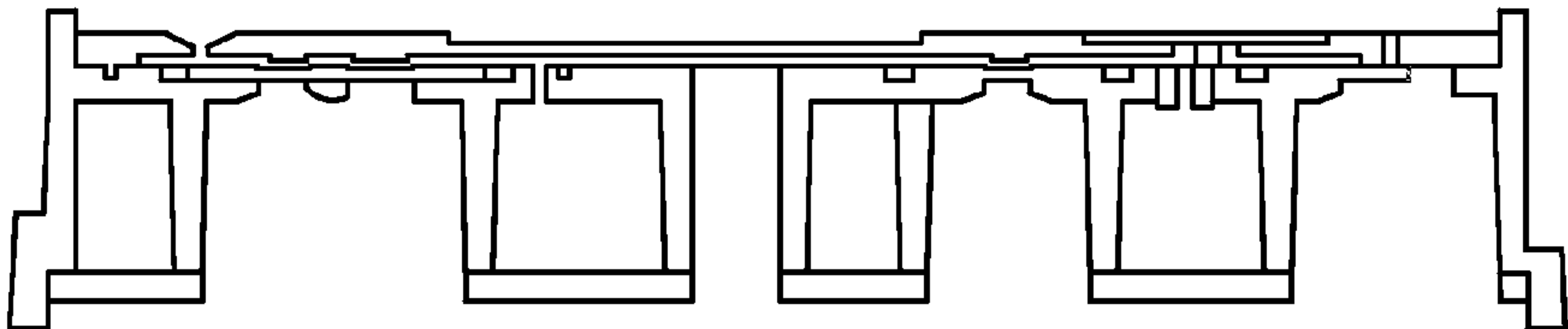


FIG. 8C



SIDE VIEW

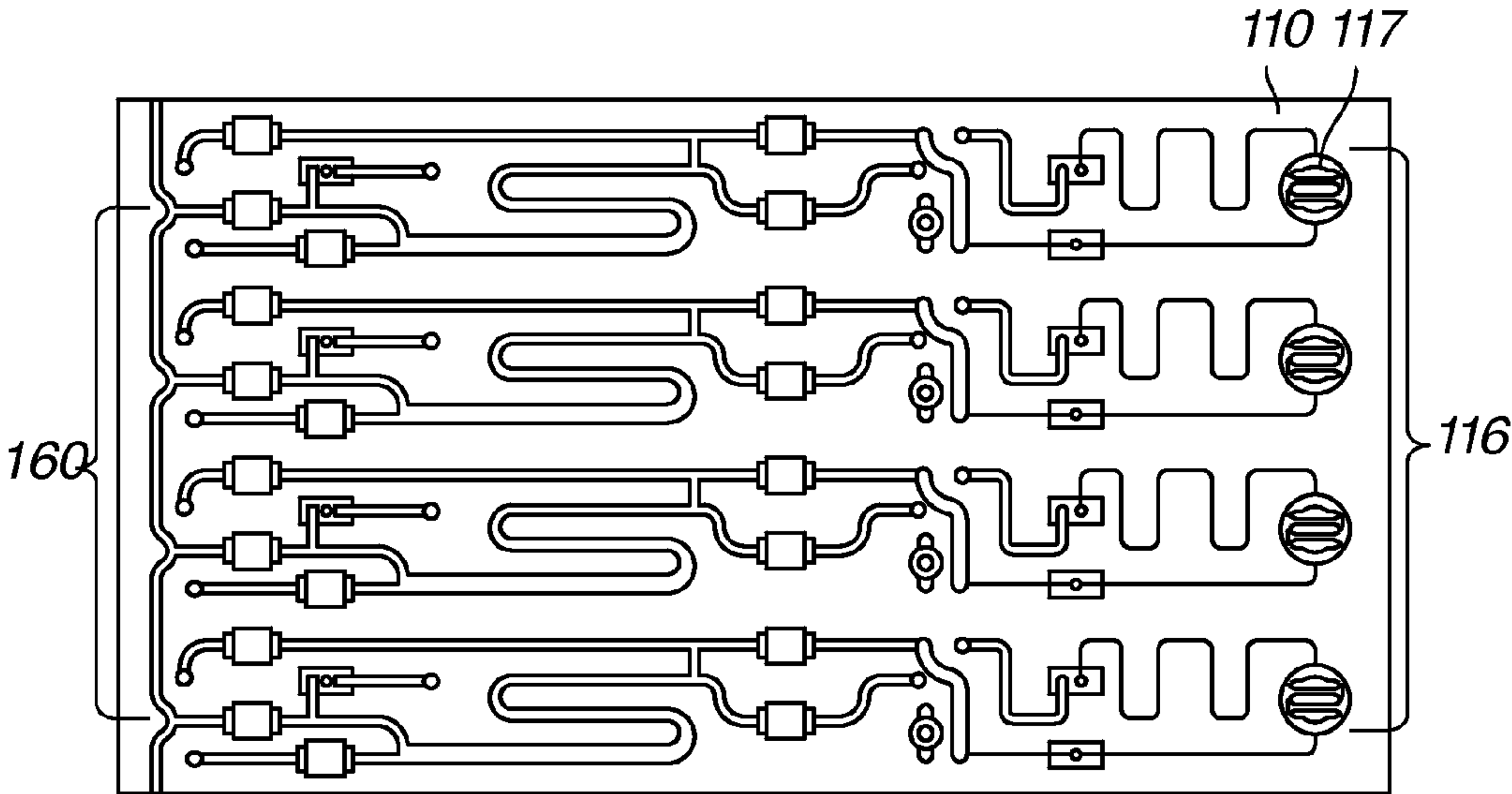


FIG. 9
TOP VIEW

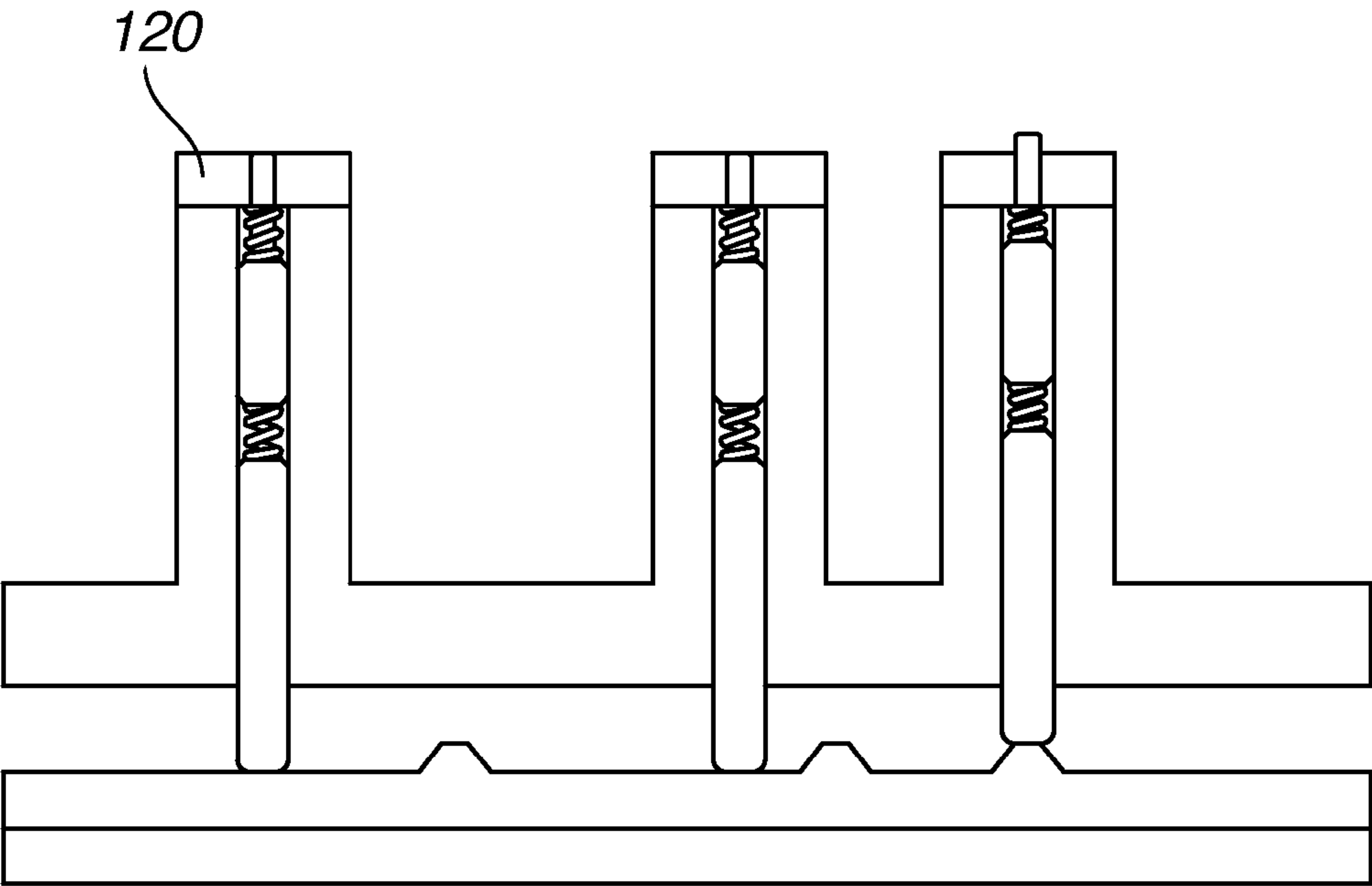


FIG. 10A

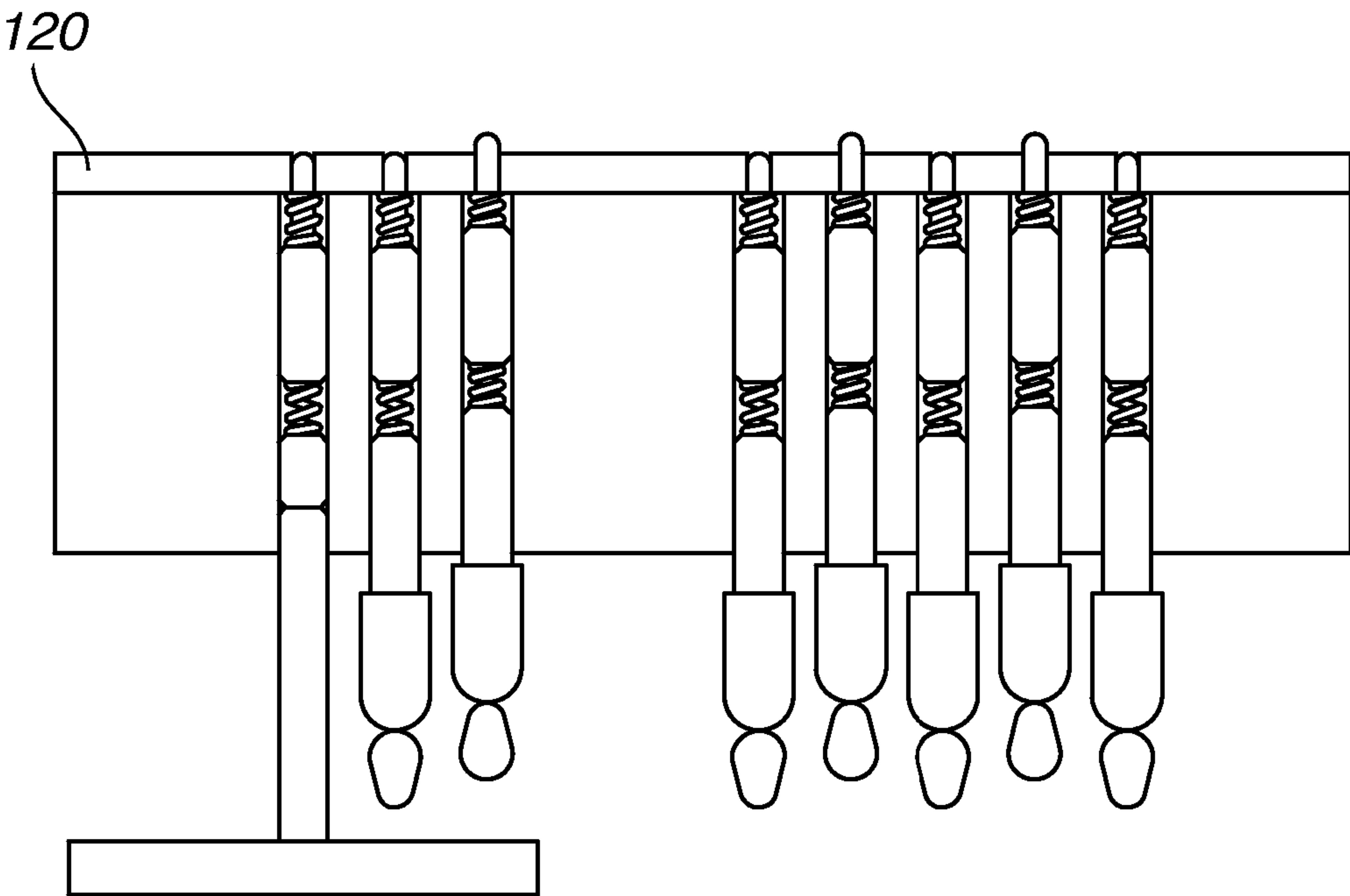


FIG. 10B

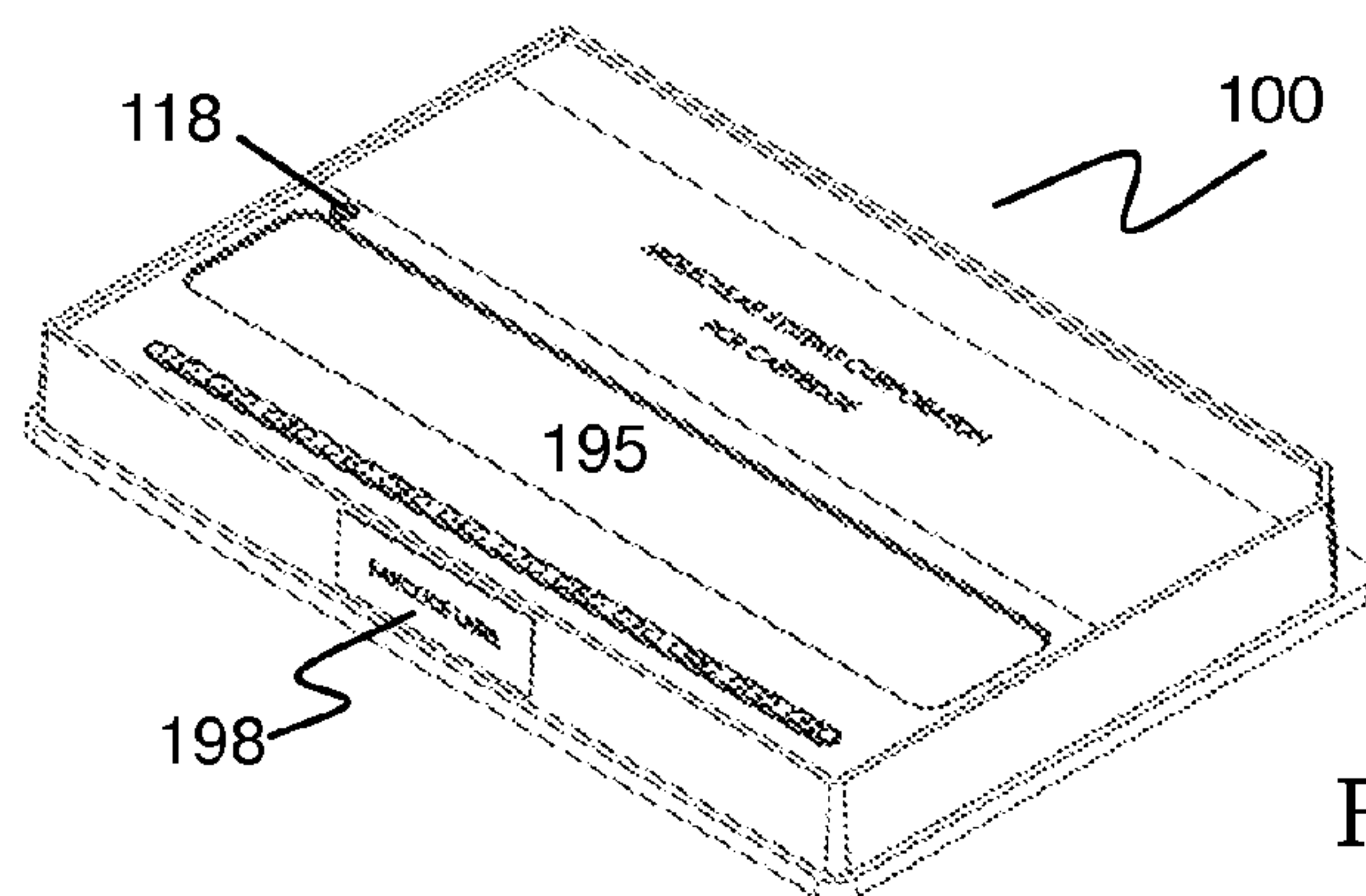


FIG. 11A

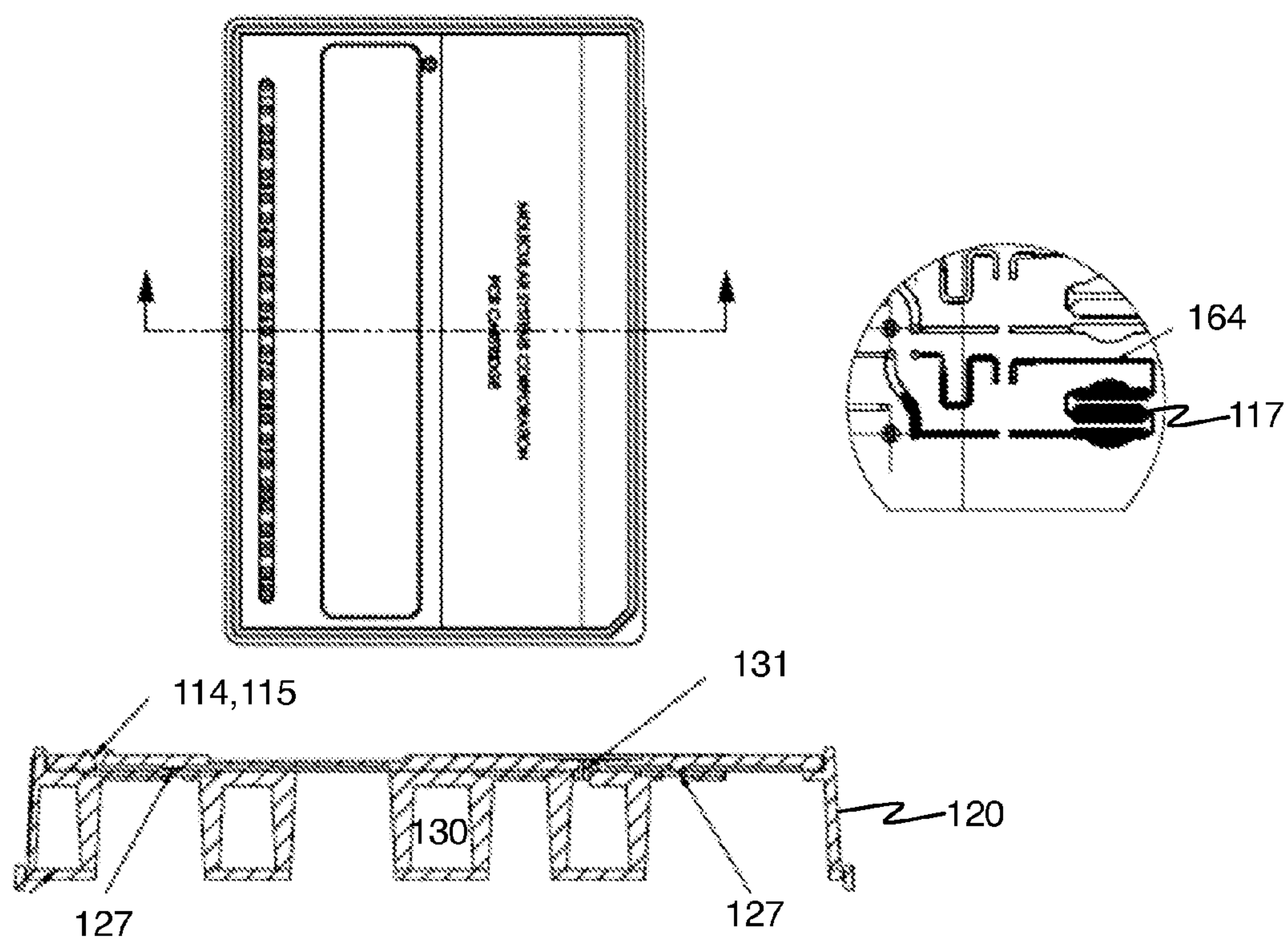


FIG. 11B

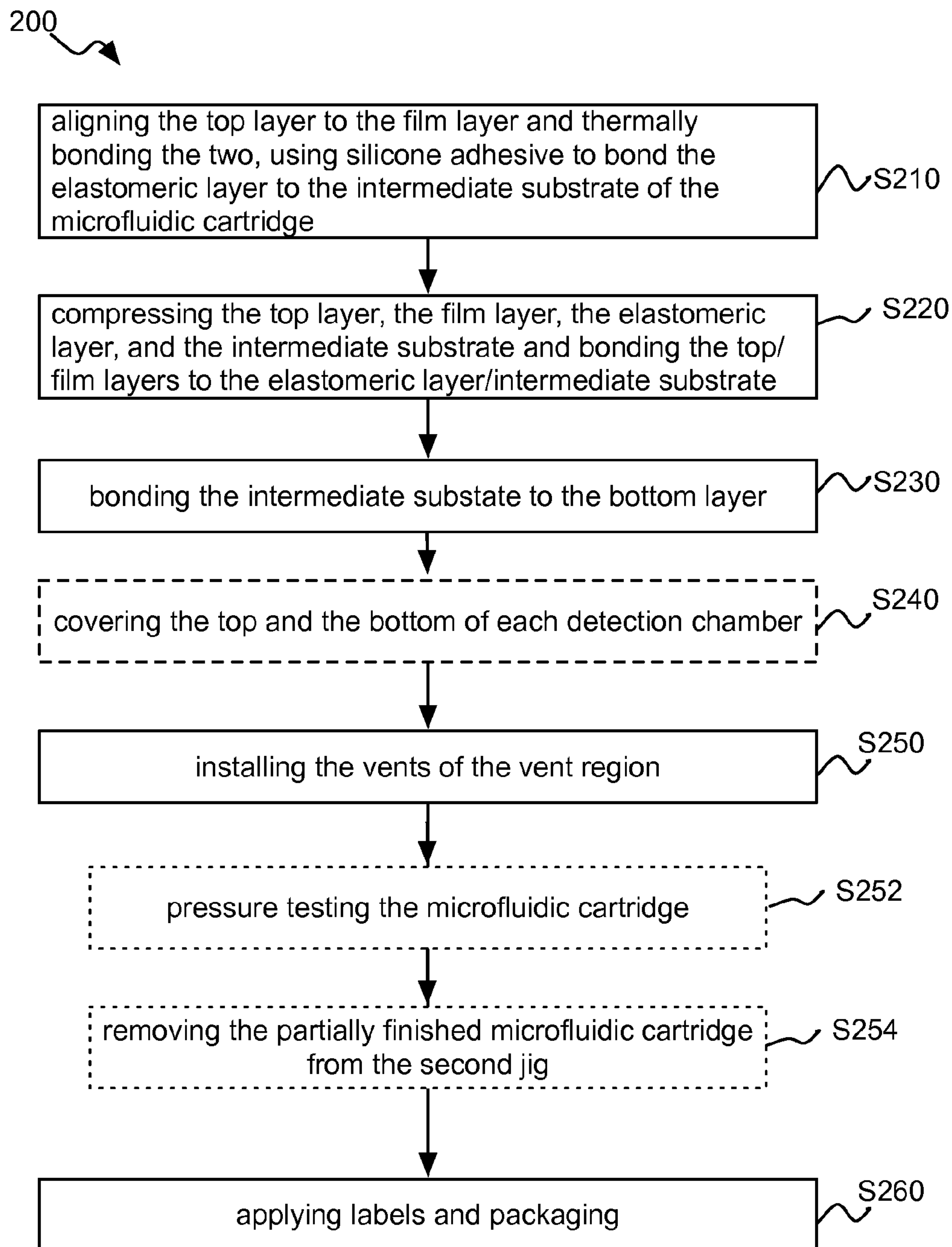


FIG. 12A

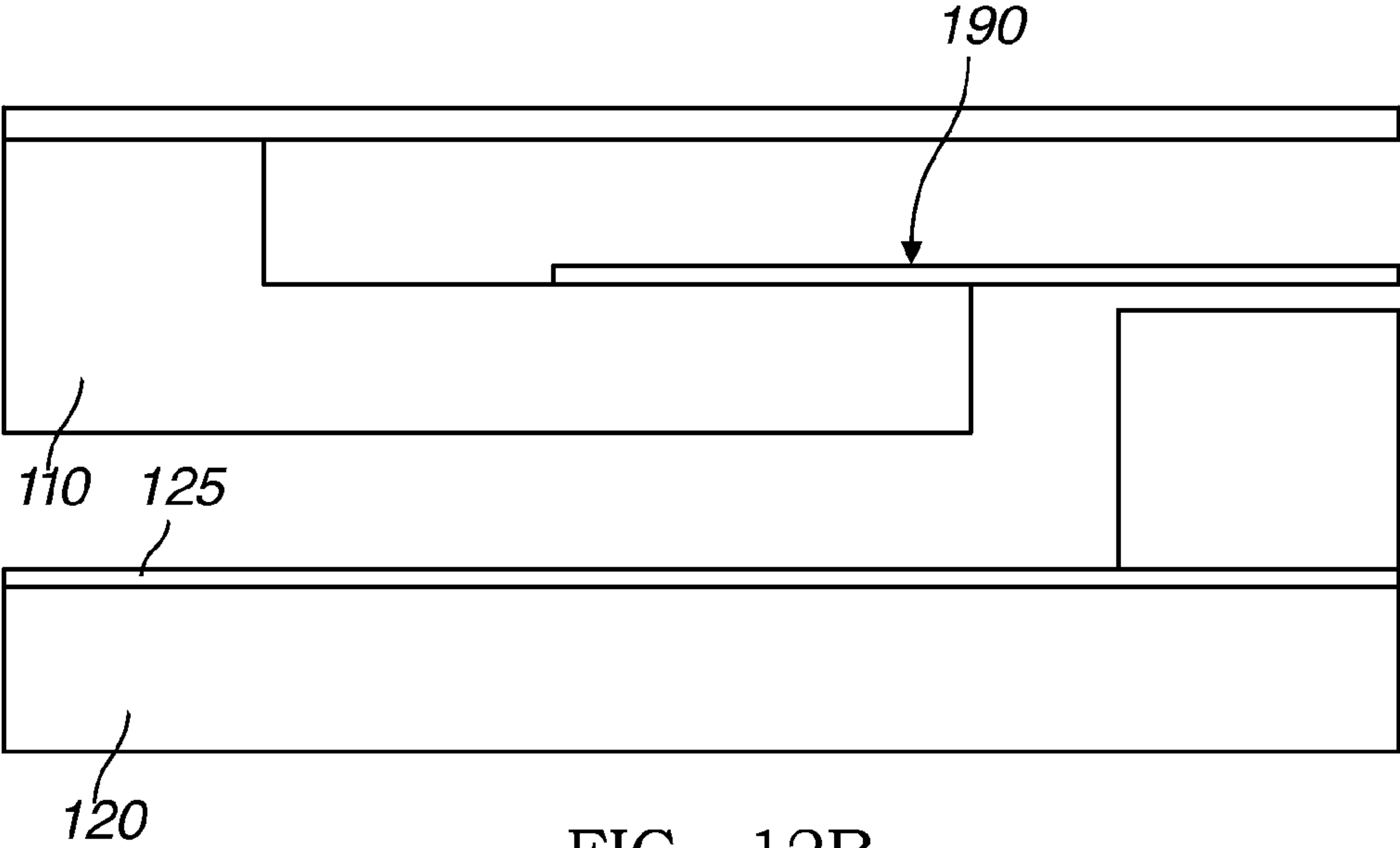


FIG. 12B

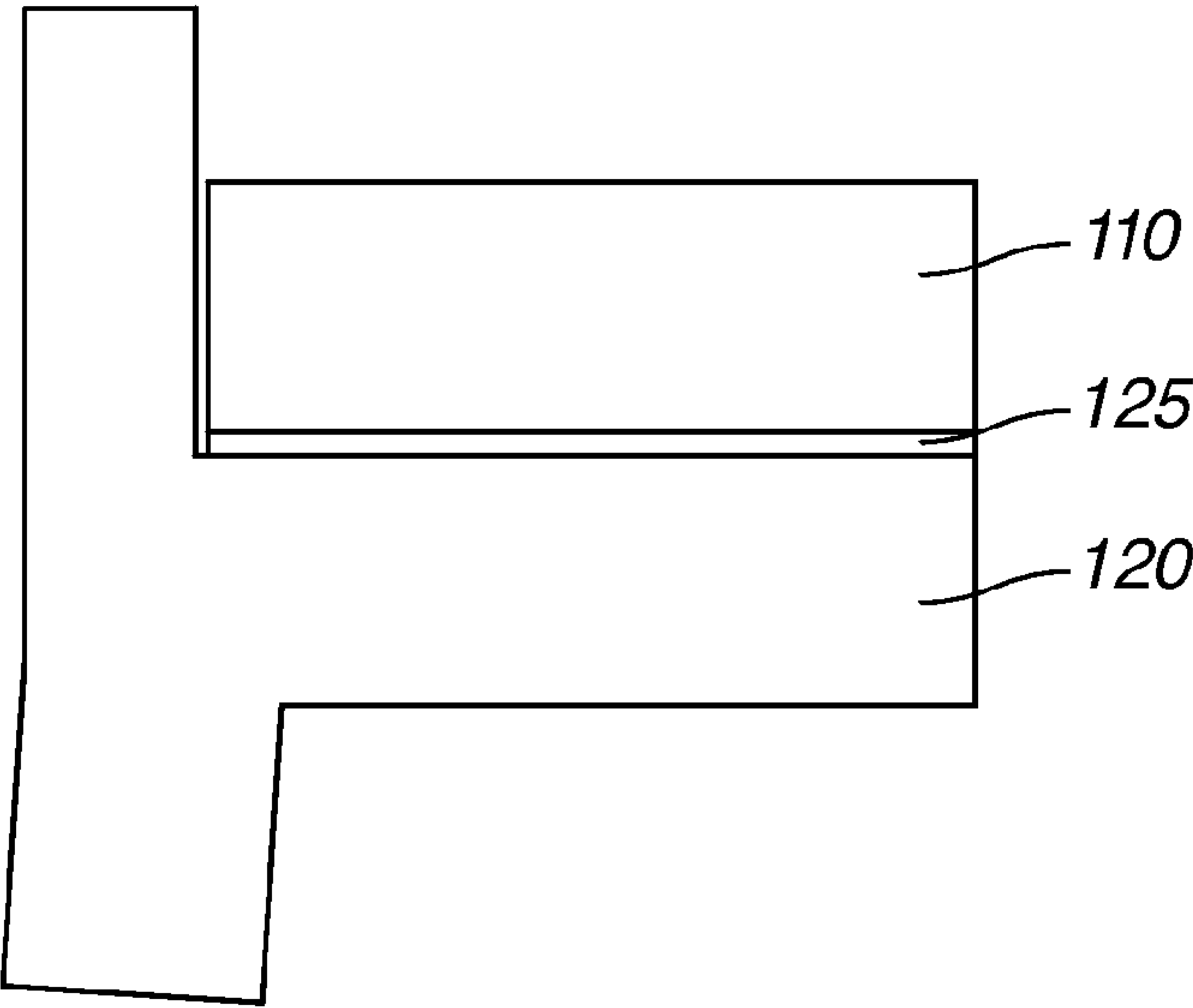


FIG. 12C

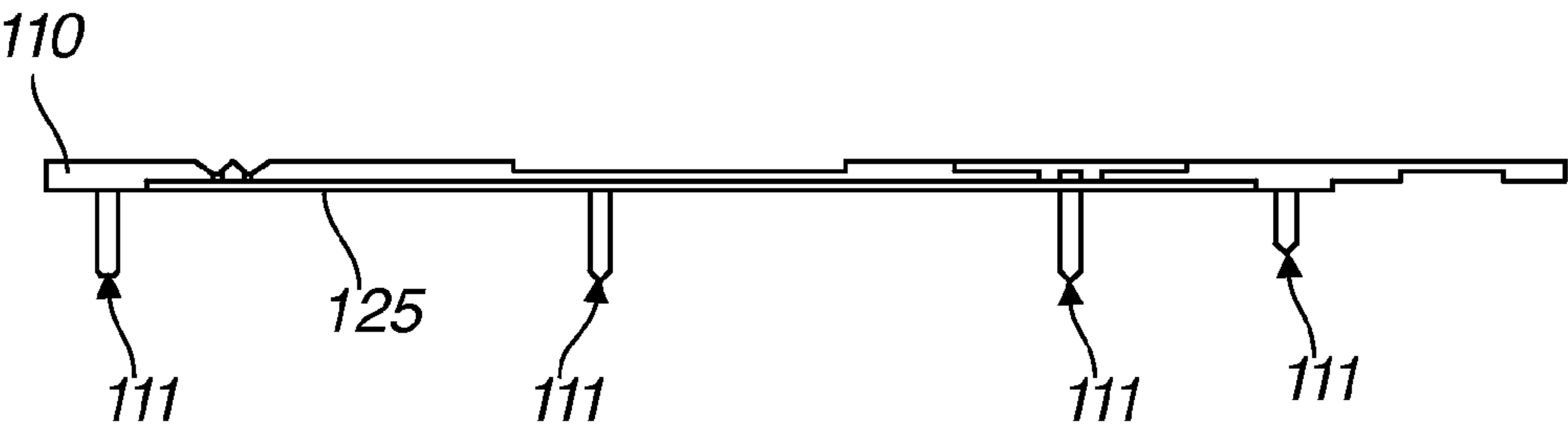


FIG. 12D

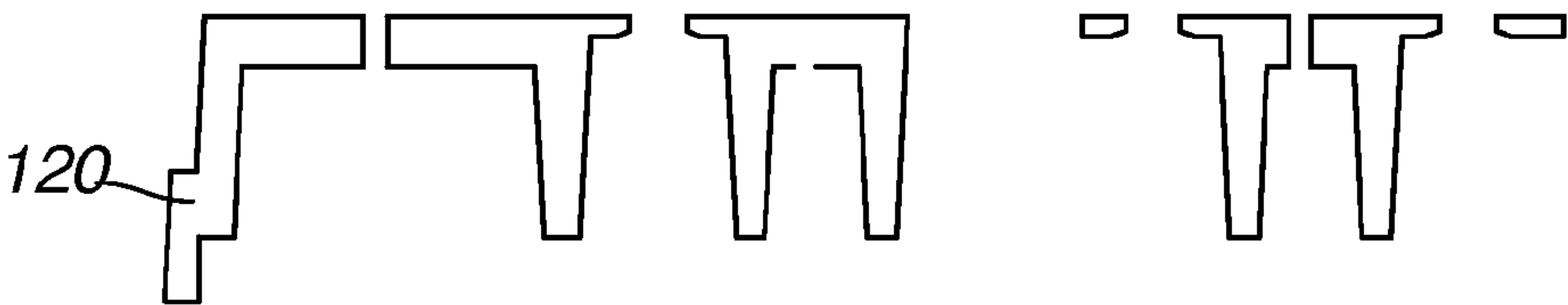


FIG. 12E

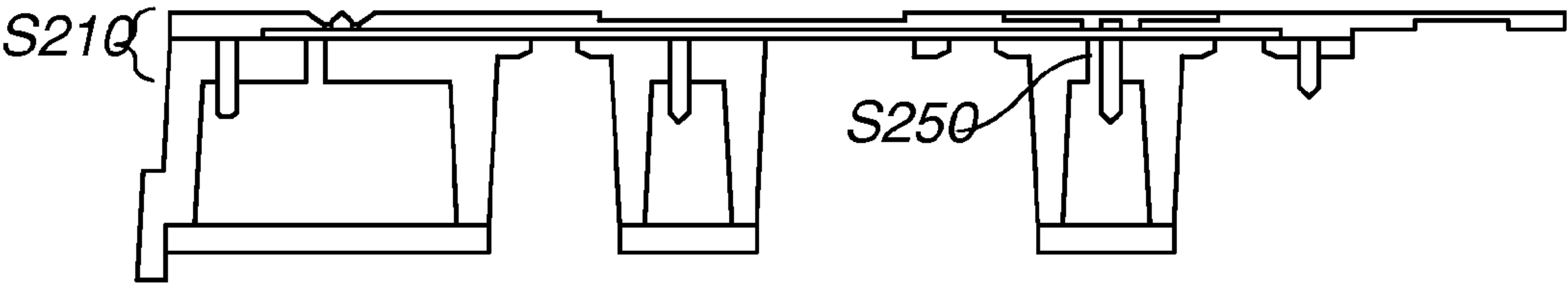


FIG. 12F

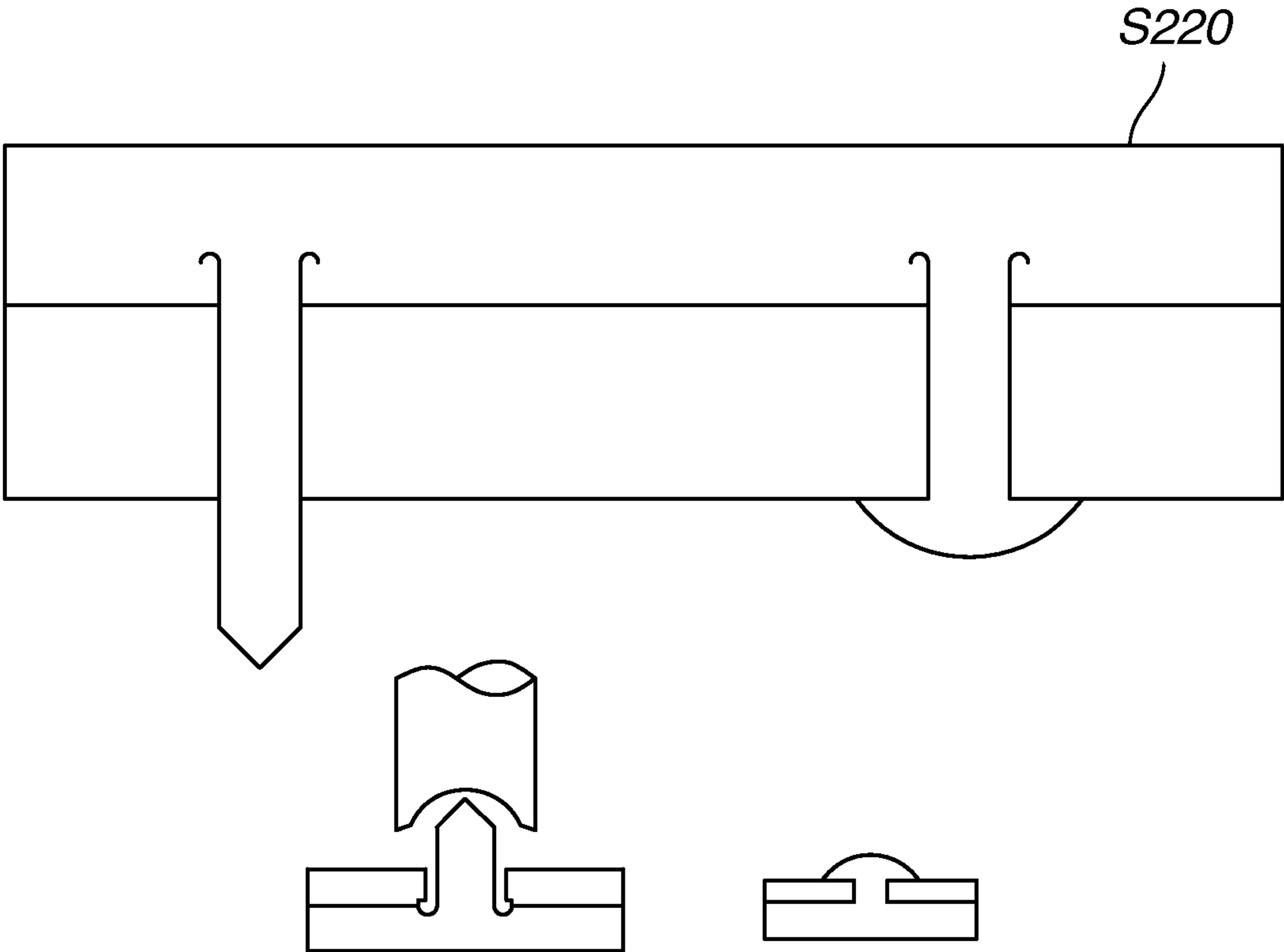


FIG. 12G

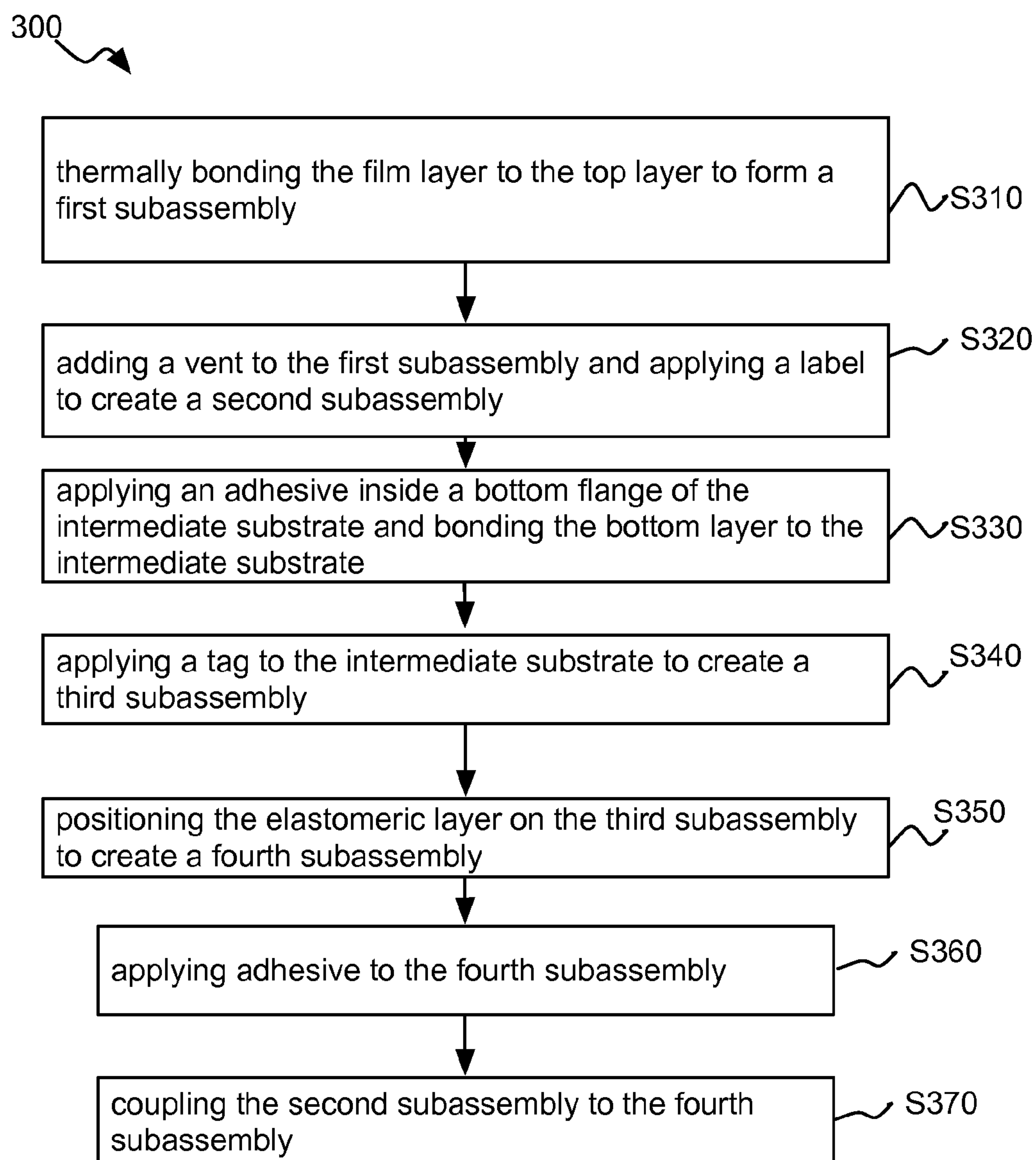


FIG. 13

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MICROFLUIDIC CARTRIDGE FOR PROCESSING AND DETECTING NUCLEIC ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 13/766,009 (now U.S. Pat. No. 9,101,930), filed 13 Feb. 2013, which claims the benefit of U.S. Provisional Application Ser. No. 61/667,606, filed on 3 Jul. 2012, and U.S. Provisional Application Ser. No. 61/598,240, filed on 13 Feb. 2012, which are all incorporated in their entirety by this reference.

TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved microfluidic cartridge for processing and detecting nucleic acids.

BACKGROUND

Molecular diagnostics is a laboratory discipline that has developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology research procedures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diagnostic use in healthcare and other fields requiring nucleic acid analysis. Molecular diagnostic analysis of biological samples can include the detection and/or monitoring of one or more nucleic acid materials present in the specimen. The particular analysis performed may be either qualitative and/or quantitative. Methods of analysis may involve isolation, purification, and amplification of nucleic acid materials, and polymerase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, and/or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and molecular diagnostic techniques are also labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient. Furthermore, methods of isolation, processing, and amplification are often specific to certain nucleic acid types and not applicable across multiple acid types. Due to these and other deficiencies of current molecular diagnostic systems and methods, there is thus a need for improved devices for processing and amplifying nucleic acids. Thus, there is a need in the molecular diagnostics field to create an improved microfluidic cartridge to facilitate processing and detecting of nucleic acids. This invention provides such a microfluidic cartridge.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1C depict an embodiment of a microfluidic cartridge (top and side views) and an embodiment of a microfluidic pathway of the microfluidic cartridge;

FIGS. 1D-K depict an example embodiment of subsets of occlusion positions defining truncated portions of a fluidic pathway;

FIG. 2 depicts an alternative embodiment of a microfluidic cartridge (top view) showing individual waste chambers located on the top of cartridge and multiple fluid ports;

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FIG. 3 depicts an alternative embodiment of a detection chamber of the microfluidic cartridge (top view) and a heating element configured to heat the detection chamber;

FIG. 4 depicts an embodiment of a waste chamber of the microfluidic cartridge;

FIGS. 5A-5D depict embodiments of the elastomeric layer of the microfluidic cartridge, in open and occluded configurations;

FIGS. 6A-6C depict an alternative embodiment of a microfluidic cartridge (top and side views) and an alternative embodiment of a microfluidic pathway of the microfluidic cartridge;

FIG. 7 depicts another alternative embodiment of a microfluidic pathway of the microfluidic cartridge;

FIGS. 8A and 8B depict schematics of microfluidic channel cross sections;

FIG. 8C depicts specific embodiments of microfluidic channel cross sections;

FIG. 9 depicts an embodiment of the microfluidic cartridge with twelve fluidic pathways (four of which are shown);

FIGS. 10A and 10B depict embodiments of occlusion of fluidic pathways with the elastomeric layer and a valving mechanism;

FIGS. 11A and 11B depict an embodiment of the microfluidic cartridge;

FIGS. 12A-12G depict an example manufacturing method for an embodiment of the microfluidic cartridge; and

FIG. 13 depicts an alternative example manufacturing method for an embodiment of the microfluidic cartridge.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description of preferred embodiments of the invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

1. Microfluidic Cartridge

As shown in FIGS. 1A-1C, an embodiment of a microfluidic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample port-reagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; and a set of fluidic pathways 160, each formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140. In other embodiments, the microfluidic cartridge 100 may further comprise a bottom layer 170 coupled to the intermediate substrate 120 and configured to seal the waste chamber 130. Furthermore, the top layer 110 of the microfluidic cartridge 100 may further comprise a shared fluid port 118, a vent region 190, and a heating region 195, such that each fluidic pathway 165 in the set of fluidic pathways 160 is fluidically coupled to a sample port-reagent port pair 113, the shared fluid port 118, the waste chamber 130, and a detection chamber 117, comprises a capture segment 166 configured to pass through the heating region and the magnetic field, and is configured to pass through the vent region 190 upstream of the detection chamber 117. Each fluidic pathway 165 thus functions to receive and facilitate processing of a sample fluid containing nucleic acids as it passes through different portions of the fluidic pathway 165. As configured, the microfluidic cartridge 100 can be used to facilitate molecular diag-

nostic processes and techniques, and preferably conforms to microtiter plate dimensional standards. Alternatively, the microfluidic cartridge **100** may be any appropriate size. In a specific application, the microfluidic cartridge **100** can be used to facilitate a PCR procedure for analysis of a sample containing nucleic acids.

1.1 Microfluidic Cartridge—Top Layer

The top layer **110** of an embodiment of the microfluidic cartridge **100** functions to accommodate elements involved in performing a molecular diagnostic procedure (e.g. PCR), such that a sample containing nucleic acids, passing through the cartridge, can be manipulated by the elements involved in performing the molecular diagnostic procedure. The top layer **110** is preferably composed of a structurally rigid/stiff material with low autofluorescence, such that the top layer **110** does not interfere with sample detection by fluorescence or chemiluminescence techniques, and an appropriate glass transition temperature and chemical compatibility for PCR or other amplification techniques. Preferably, the top layer **110** is composed of a polypropylene-based polymer, but the top layer **110** may alternatively be composed of any appropriate material (e.g. cyclic olefin polymer). In a specific embodiment, the top layer **110** is composed of 1.5 mm thick polypropylene produced by injection molding, with a glass transition temperature between 136 and 163° C. The top layer **110** may alternatively be composed of any appropriate material, for example, a polypropylene based polymer. As shown in FIGS. **1B** and **1C**, the top layer **110** preferably comprises a set of sample port-reagent port pairs **112**, a fluid port **118**, a vent region **190**, a heating region **195** crossing a capture segment **166** of a fluidic pathway **165**, and a set of detection chambers **116**.

Each sample-port-reagent port pair **113** of an embodiment of the top layer **110** comprises a sample port **114** and a reagent port **115**. The sample port **114** functions to receive a volume of a sample fluid potentially containing the nucleic acids of interest for delivery of the volume of fluid to a portion of a fluidic pathway **165** coupled to the sample port-reagent port pair **113**. In a specific embodiment, the volume of a sample fluid is a biological sample with magnetic beads for nucleic acid isolation; however, the volume of fluid comprising a sample fluid may alternatively be any appropriate fluid containing a sample with nucleic acids. Preferably, each sample port **114** is isolated from all other sample ports, in order to prevent cross-contamination between samples of nucleic acids being analyzed. Additionally, each sample port **114** is preferably of an appropriate geometric size and shape to accommodate a standard-size pipette tip used to deliver the volume of a sample fluid without leaking. Alternatively, all or a portion of the sample ports **114** are configured to be coupled to fluid conduits or tubing that deliver the volume of a sample fluid.

Each sample-port reagent port pair **113** of an embodiment of the top layer **110** also comprises a reagent port **115**, as shown in FIG. **1A**. The reagent port **115** in a sample port-reagent port pair **113** functions to receive a volume of fluid comprising a reagent used in molecular diagnostics, for delivery of the volume of fluid comprising a reagent to a portion of a fluidic pathway **165** coupled to the sample port-reagent port pair **113**. In a specific embodiment, the volume of fluid comprising a reagent used in molecular diagnostics is a sample of reconstituted molecular diagnostic reagents mixed with nucleic acids released and isolated using the microfluidic cartridge **100**; however, the volume of fluid comprising a reagent used in molecular diagnostics may alternatively be any appropriate fluid comprising reagents used in molecular diagnostics. Preferably, each reagent port **115** is isolated from

all other reagent ports, in order to prevent cross-contamination between samples of nucleic acids being analyzed. Additionally, each reagent port **115** is preferably of an appropriate geometric size to accommodate a standard-size pipette tip used to deliver the volume of fluid comprising a reagent used in molecular diagnostics. Alternatively, all or a portion of the reagent ports **115** are configured to be coupled to fluid conduits or tubing that deliver the volume of fluid comprising a reagent used in molecular diagnostics.

Preferably, the set of sample port-reagent port pairs **112** is located near a first edge of the top layer **110**, such that the configuration of the sample port-reagent port pairs **112** functions to increase accessibility, for instance, by a pipettor delivering fluids to the microfluidic cartridge **100**. In one specific example, the microfluidic cartridge **100** is configured to be aligned within a module, with the set of sample port-reagent port pairs **112** accessible outside of the module, such that a multichannel pipette head can easily access the set of sample port-reagent port pairs **112**. Preferably, as shown in FIG. **1A**, the set of sample port-reagent port pairs **112** is configured such that the sample ports **114** and the reagent ports **115** alternate along the first edge of the top layer **110**. In an alternative embodiment, the set of sample port-reagent port pairs **112** may not be located near an edge of the top layer **110**, and may further not be arranged in an alternating fashion.

The fluid port **118** of the top layer **110** of the microfluidic cartridge functions to receive at least one of a wash fluid, a release fluid, and a gas used in a molecular diagnostic procedure, such as PCR. In an embodiment, the wash fluid, the release fluid, and/or the gas are common to all samples being analyzed during a run of the diagnostic procedure using the microfluidic cartridge **100**; in this embodiment, as shown in FIG. **1A**, the fluid port **118** is preferably a shared fluid port, fluidically coupled to all fluidic pathways **165** coupled to the sample port-reagent port pairs **112**, and configured to deliver the same wash fluid, release fluid, and/or gas through the shared fluid port. Alternatively, as shown in FIG. **2**, the top layer may comprise more than one fluid port **118**, configured to deliver different wash fluids, release fluids, and/or gases to individual or multiple fluidic pathways **165** coupled to the set of sample port-reagent port pairs **112**.

Preferably, the fluid port **118** is located along an edge of the microfluidic cartridge **100**, which functions to increase accessibility to the fluid port by a system delivering fluids to the fluid port **118**. In a specific embodiment, as shown in FIG. **1A**, the fluid port is located approximately midway along an edge of the microfluidic cartridge **100**, different from the edge along which the set of sample port-reagent port pairs **112** is located. Alternatively, the fluid port **118** may not be located along an edge of the microfluidic cartridge **100**. Additionally, the fluid port **118** is preferably configured to be coupled to a syringe pump for fluid delivery; however, the fluid port **118** may alternatively be configured to couple to any appropriate system for fluid delivery. Preferably, the wash fluid is a wash buffer for washing bound nucleic acid samples (i.e. nucleic acids bound to magnetic beads), the release fluid is a reagent for releasing bound nucleic acids samples from the magnetic beads, and the gas is pressurized air for moving fluids and demarcating separate reagents. Alternatively, the wash fluid, release fluid, and gas may be any appropriate liquids or gases used to carry out a molecular diagnostic procedure.

The heating region **195** of the top layer **110** functions to accommodate and position a heating element relative to elements of the microfluidic cartridge **100**. The heating element preferably heats a defined volume of fluid and the magnetic beads, which has traveled through the microfluidic cartridge

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100, according to a specific molecular diagnostic procedure protocol (e.g. PCR protocol), and is preferably an element external to the microfluidic cartridge 100; alternatively, the heating element may be integrated with the microfluidic cartridge and/or comprise a thermally conductive element integrated into the microfluidic cartridge 100. The heating region 195 is preferably a recessed fixed region of the top layer 110, downstream of the sample port-reagent port pairs 112, as shown in FIGS. 1A and 1B. Alternatively, the heating region may not be fixed and/or recessed, such that the heating region 195 sweeps across the top layer 110 of the microfluidic cartridge 100 as the heating element is moved. The microfluidic cartridge 100 may altogether omit the heating region 195 of the top layer 110, in alternative embodiments using alternative processes (e.g. chemical methods) for releasing nucleic acids from nucleic acid-bound magnetic beads.

The vent region 190 of an embodiment of the top layer 110 functions to remove unwanted gases trapped within a fluidic pathway 165 of the microfluidic cartridge, and may additionally function to position a defined volume of fluid within a fluidic pathway 165 of the microfluidic cartridge. The vent region 190 is preferably located downstream of the heating region 195 in an embodiment where the heating region 195 is fixed on the top layer 110 of the microfluidic cartridge 100, but alternatively may be located at another appropriate position on the top layer 110 such that unwanted gases are substantially removed from the microfluidic cartridge 100 during analysis. The top layer 110 may alternatively comprise more than one vent region 190 located at appropriate positions in the top layer 110. Preferably, as shown in FIGS. 1A and 1B, the vent region 190 is a recessed region in the top layer 110, and further comprises a film covering the vent region 190. Preferably, the film covering the vent region 190 is a gas-permeable but liquid-impermeable film, such that unwanted gases may be released from the microfluidic cartridge 100, but fluids remain within the microfluidic cartridge 100 and flow to the point of contacting the film. This functions to remove unwanted gases and position a defined volume of fluid within a fluidic pathway 165 of the microfluidic cartridge. In a specific embodiment, the film covering the vent region is a hydrophobic porous polytetrafluoroethylene-based material, synthesized to be gas-permeable but liquid-impermeable. Alternatively, the film covering the vent region may be gas and liquid permeable, such that unwanted gases and liquids are expelled from the microfluidic cartridge 100 through the vent region 190. Other alternative embodiments of the microfluidic cartridge 100 may altogether omit the vent region.

The set of detection chambers 116 of an embodiment of the top layer no functions to receive a processed nucleic acid sample, mixed with molecular diagnostic reagents, for molecular diagnostic analysis. Preferably, the set of detection chambers 116 is located along an edge of the top layer 110, opposite the edge along which the set of sample port-reagent port pairs 112 is located, which allows sample fluids dispensed into the microfluidic cartridge 100 to be processed and mixed with molecular diagnostic reagents on their way to a detection chamber 117 of the set of detection chambers 116 and facilitates access to the detection chambers by external elements performing portions of a molecular diagnostics protocol (e.g. heating and optics systems). Alternatively, the set of detection chambers 116 may not be located along an edge of the top layer 110. In a first variation, as shown in FIGS. 1A and 11B, each detection chamber 117 in the set of detection chambers comprises a serpentine-shaped channel 16 for facilitating analysis of a solution of nucleic acids mixed with reagents. In the first variation, three portions of the serpen-

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tine-shaped channel 16 are preferably wide and shallow to facilitate heating, and are interconnected by two narrow portions, which function to increase fluid flow resistance and reduce the proportion of nucleic acid not contained within the detection area. The first variation functions to facilitate filling of the set of detection chambers in a manner that reduces the potential for trapped air bubbles, to facilitate rapid molecular diagnostic techniques, and to comply with current imaging technologies. In a specific example of the first variation, each serpentine-shaped channel 16 is injected molded into the top layer 110 of the microfluidic cartridge 100, and the three interconnected portions of the serpentine-shaped channel 16 are each 1600 μm wide by 400 μm deep.

In a second variation, each detection chamber 117 in the set of detection chambers has a depth between 0.400 mm and 1.00 mm, and a diameter between 3.50 mm and 5.70 mm, to provide a volumetric configuration that facilitates reaction efficiency. In a specific example of the second variation, each detection chamber 117 in the set of detection chambers 116 is configured to contain a total volume of 10 μL , and has a depth of 0.80 mm and a diameter of 3.99 mm; however, in alternative embodiments, each detection chamber 117 in the set of detection chambers 116 may be configured to contain a total volume less than or greater than 10 μL .

Preferably, as shown in FIGS. 1A and 1B, the lower regions of each detection chamber 117 in the set of detection chambers 116 includes a PCR compatible film that is thin, to facilitate efficient thermocycling, and has low autofluorescence, to facilitate light-based molecular diagnostic assays performed at the set of detection chambers 116. The PCR compatible film is preferably composed of a polypropylene based polymer thermally bonded to the bottom of the top layer, but may alternatively be composed of any appropriate PCR-compatible material and bonded in any fashion. In one specific variation, the PCR compatible film is a cyclic olefin polymer (COP) film, thermally bonded to the top layer 110, with a glass transition temperature suitable for a molecular diagnostic protocol. In one alternative embodiment, depending on the configuration of imaging, heating, and/or cooling elements external to the microfluidic cartridge 100, the top and/or bottom of the detection chambers 117 in the set of detection chambers 116 may be entirely formed of a clear or transparent material (e.g. glass or plastic) allowing transmission of light. In a variation of this alternative embodiment, lensing, other optical components, or additional structures may also be incorporated into the detection chambers, to facilitate light transmission and/or focusing. In the variation of the alternative embodiment, a lens may be manufactured (e.g. injection molded) directly to form a surface of a detection chamber 117.

In the embodiment of the set of detection chambers 116 that includes a PCR compatible film, the PCR compatible film may further include a thermally conductive component, which functions to transfer heat from a heating element to the detection chamber. Depending on the position of the heating element(s) relative to the microfluidic cartridge 100 during analysis, the thermally conductive component of the PCR compatible film may be integrated with just the upper region of each detection chamber, just the lower region of each detection chamber, or both the upper and lower regions of each Detection chamber. The thermally conductive component of the PCR compatible film may comprise a wire mesh with a substantially small wire diameter, as shown in FIG. 3, thermally conductive particles distributed through the PCR compatible film (in a manner that still allows for optical clarity), or any other appropriate thermally conductive component (e.g. thermally conductive beads integrated into the

PCR compatible film). The region laterally around the detection chamber may also further include one or more heat-transfer elements or air channels speed heat dissipation. Alternatively, a detection chamber **117** in the set of detection chambers **116** may not include a PCR compatible film with a thermally conductive component. Preferably, each detection chamber **117** is heated using a diced silicon wafer with conductive channels flip-chip bonded to a detection chamber to provide resistive heating; however, each detection chamber **117** may alternatively be heated using any appropriate heating device or method, and may be assembled using any appropriate method.

Preferably, each detection chamber **117** in the set of detection chambers **116** is thermally isolated from all other detection chambers, in order to prevent contamination of data from a detection chamber **117** due to heat transfer from other detection chambers in the set of detection chambers **116**. In one embodiment, each detection chamber **117** of the set of detection chambers **116** is spaced far from adjacent detection chambers to limit thermal crosstalk. In another alternative embodiment, the top layer **110** may comprises slots between adjacent detection chambers to separate the detection chambers with an air gap. In one variation, thermal isolation is achieved by surrounding the side walls of each detection chamber **117** with a thermally insulating material, such as an insulating epoxy, putty, filler, or sealant. In another variation, the thermally insulating material has a low density, which functions to reduce heat transfer from other detection chambers. In yet another variation, thermal isolation is achieved by geometrically separating or displacing the detection chambers relative to each other within the top layer **110** of the microfluidic cartridge **100**, such that heat transfer between detection chambers is hindered.

Preferably, each detection chamber **117** in the set of detection chambers **116** is also optically isolated from all other detection chambers, in order to prevent contamination of data from a detection chamber **117** due to light transfer from other detection chambers in the set of detection chambers **116**. Preferably, optical isolation is achieved with detection chambers having substantially vertical walls, and separating each detection chamber **117** in the set of detection chambers from each other. However, in one variation, the sidewalls of each detection chamber **117** in the set of detection chambers **116** are either composed of or surrounded by a material with low autofluorescence and/or poor optical transmission properties to achieve optical isolation. In another variation, the sidewalls of each detection chamber **117** are surrounded by an optically opaque material, thus allowing transmission of light to a detection chamber **117** through only the top and bottom regions of the detection chamber **117**. Alternatively, the microfluidic cartridge **100** may not further comprise any provisions for optical isolation of each detection chamber **117** in the set of detection chambers **116**, aside from constructing the set of detection chambers **116** with a material having low autofluorescence.

Additionally, each detection chamber **117** in the set of detection chambers **116** may be further optimized to meet volumetric capacity requirements, facilitate high thermocycling rates, facilitate optical detection, and facilitate filling in a manner that limits bubble generation. Alternatively each detection chamber **117** in the set of detection chambers **116** may not be optimized to meet volumetric capacity requirements, facilitate high thermocycling rates, facilitate optical detection, and/or facilitate filling in a manner that limits bubble generation.

The top layer **110** of the microfluidic cartridge **100** may further comprise a set of cartridge-aligning indentations **180**,

which function to align the microfluidic cartridge **100** as it moves through an external module. As shown in FIG. **2** the set of cartridge-aligning indentations **180** are preferably located such that they do not interfere with any ports **112**, **118**, the heating region, **195**, the vent region **190**, and/or the set of detection chambers **116**. In an embodiment, the top layer **110** of the microfluidic cartridge preferably comprises at least four cartridge-aligning indentations, located at points on the periphery of the top layer **110**, and the cartridge-aligning indentations are configured to be recessed regions configured to mate with alignment pins in a system external to the microfluidic cartridge **100**. Alternatively, the cartridge-aligning indentations may be grooves, such that the microfluidic cartridge **100** accurately slides into position along the grooves within a system external to the microfluidic cartridge **100**. In yet another alternative embodiment, the set of cartridge-aligning indentations **180** may be any appropriate indentations that allow for positioning of the microfluidic cartridge **100** within an external system. However, the microfluidic cartridge **100** may altogether omit the set of cartridge-aligning indentations **180**, and rely upon other features of the microfluidic cartridge **100** to facilitate alignment.

1.2 Microfluidic Cartridge—Intermediate Substrate

As shown in FIG. **1B**, an embodiment of the microfluidic cartridge also comprises an intermediate substrate **120**, coupled to the top layer **110** and partially separated from the top layer **110** by a film layer **125**, configured to form a waste chamber **130**. The intermediate substrate **120** functions to serve as a substrate to which layers of the microfluidic cartridge may be bonded, to provide guides for the valve pins, and to provide a waste chamber volume into which a waste fluid may be deposited. Preferably, the depth of the intermediate substrate **120** provides a waste chamber volume adequate to accommodate the volume of waste fluids generated within the microfluidic cartridge **100**. Additionally, the depth of the intermediate substrate **120** provides a low profile for the microfluidic cartridge **100** to facilitate movement throughout a compact molecular diagnostic system. Preferably, the intermediate substrate **120** of the microfluidic cartridge **100** is also configured such that the footprint of microfluidic cartridge **100** adheres to microtiter plate standards, to facilitate automated handling of the microfluidic cartridge **100**. The intermediate substrate **120** is preferably composed of a low-cost, structurally stiff material, such as polypropylene. However, similar to the top layer **120**, the intermediate substrate may be alternatively composed of a structurally stiff material with low autofluorescence, such that the intermediate substrate **120** does not interfere with sample detection by fluorescence techniques, and an appropriate glass transition temperature for PCR techniques. In one variation of this alternative embodiment, the intermediate substrate **120** is composed of a cyclic olefin polymer (COP), produced by injection molding, with a glass transition temperature between 136 and 163° C. In yet another alternative embodiment, the intermediate substrate **120** may be composed of any appropriate material, for example, a polycarbonate based polymer.

Preferably, the intermediate substrate **120** of the microfluidic cartridge **100** is coupled to the top layer **110** and partially separated from the top layer **110** by a film layer **125**. The film layer **125** functions to isolate individual fluidic pathways **165** of the microfluidic cartridge, to prevent leakage, to provide an appropriate environment for sample processing and conducting a molecular diagnostic protocol, and to provide access between a microfluidic channel (of a fluidic pathway **165**) above the film layer **125** and elements below the film layer **125** (e.g. waste chamber and/or fluidic pathway occluder).

Preferably, the film layer is a polypropylene (PP) with an appropriate glass transition temperature, such that it is PCR compatible and thermally bondable to the top layer 110; however, the film layer may alternatively be any appropriate material. In a specific embodiment, the film layer 125 is a polypropylene film between 30 and 100 microns thick and die cut to produce openings at a set of occlusion positions, to provide access between a microfluidic channel of a fluidic pathway 165 above the film layer 125 and elements below the film layer 125. In this specific embodiment, the openings are slightly oversized prior to assembly, in order to allow for constriction during assembly (due to thermal and pressure effects) and to provide higher tolerance during assembly of microfluidic cartridge layers. Alternatively, the film layer is any appropriate material such that it substantially isolates individual fluidic pathways, and is easily processable to provide access between a microfluidic channel of a fluidic pathway 165 above the film layer and elements below the film layer 125.

Preferably, the top layer 110, the film layer 125, and the intermediate substrate are bonded together, such that the top layer 110, film layer, 125, and intermediate substrate form a bonded unit with a hermetic seal to prevent fluid leakage. A hermetic seal is preferably formed using a silicone rubber layer coupled to the film layer 125, but may alternatively be formed using an alternative material or method. In a specific embodiment, a hermetic seal formed using a silicone rubber layer is only required at locations of openings within the film layer (e.g., at locations where an external occluder interacts with the microfluidic cartridge). Preferably, in an embodiment where the top layer 110, the film layer 125, and the intermediate substrate 120 are substantially identical materials (e.g. polypropylene), at least one of thermal bonding, adhesives, and ultrasonic welding are used to coupled the layers 110, 125, 120 together. In an embodiment where the top layer 110, the film layer 125, and the intermediate substrate 120 are substantially different materials—a combination of thermal bonding methods and adhesives may be used to bond the top layer 110, the film layer 125, and the intermediate substrate 120 of the microfluidic cartridge 100 together. In an alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 of the microfluidic cartridge 100 may be thermally bonded together in a single step. In yet another alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 may alternatively be modular, in applications where a portion of the microfluidic cartridge 100 is partially reusable (e.g. in an application where the waste chamber may be discarded after use, but the top layer and film may be reused). In yet another alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 may only be partially bonded, such that a molecular diagnostic system, into which the microfluidic cartridge 100 is loaded, is configured to compress the top layer 110, the film layer 125, and the intermediate substrate 120 together, preventing any fluid leakage.

As shown in FIG. 1B, the intermediate substrate 120 of an embodiment of the microfluidic cartridge 100 is configured to form a waste chamber 130, which functions to receive and isolate waste fluids generated within the microfluidic cartridge 100. The waste chamber 130 is preferably continuous and accessible by each fluidic pathway 165 of the microfluidic cartridge 100, such that all waste fluids generated within the microfluidic cartridge 100 are deposited into a common waste chamber; however, each fluidic pathway 165 of the microfluidic cartridge 100 may alternatively have its own corresponding waste chamber 130, such that waste fluids

generated within a fluidic pathway 165 of the microfluidic cartridge 100 are isolated from waste fluids generated within other fluidic pathways 165 of the microfluidic cartridge 100. In a specific embodiment of the microfluidic cartridge 100 with a continuous waste chamber, the waste chamber has a volumetric capacity of approximately 25 mL; however, the waste chamber 130 of another embodiment may have a different volumetric capacity. The intermediate substrate 120 further comprises a waste vent 135, which provides access between a microfluidic channel of a fluidic pathway 165 above the film layer 125 and the waste chamber 130. Preferably, the intermediate substrate 130 comprises more than one waste inlet 136, such that the waste chamber is accessible at more than one location along a fluidic pathway 165 through the waste inlets 136. Alternatively, the intermediate substrate 120 may include a single waste inlet 136, such that all waste fluids generated within the microfluidic cartridge 100 are configured to travel through the single waste inlet 136 into the waste chamber 130. Also, as shown in FIG. 1B, the intermediate substrate 120 may comprise a waste vent 131, such that the waste chamber 130 is vented to prevent pressure build up in the waste chamber as waste fluid is added.

As shown in FIGS. 1B and 4, the waste chamber 130 formed by the intermediate substrate 120 preferably has a corrugated surface 137, such that the waste chamber 130 is not only configured to receive and isolate a waste fluid, but also functions to 1) provide structural stability for the microfluidic cartridge 100 and 2) allow elements external to the microfluidic cartridge 100 to enter spaces formed by the corrugated surface 137, for greater accessibility to elements of the microfluidic cartridge 100. Also shown in FIGS. 1B and 4, each of the ridges in the corrugated surface 137 may not have the same dimensions, as a result of the locations of elements within and external to the microfluidic cartridge 100. In an embodiment of the waste chamber 130 with a corrugated surface 137, at least two ridges of the corrugated surface 137 are preferably the same height, such that the microfluidic cartridge 100 sits substantially level on a flat base. In an alternative embodiment, all ridges of the corrugated surface 137 of the waste chamber 130 are identical, for structural symmetry, and in yet another embodiment, the waste chamber 130 may not have a corrugated surface 137.

In one preferred embodiment, the intermediate substrate 120 of the microfluidic cartridge 100 further comprises a set of valve guides, which function to direct a series of external pins or other indenters through the valve guides at a set of occlusion positions 141, thus affecting flow through a microfluidic channel of a fluidic pathway 165 at the set of occlusion positions 141. The set of valve guides 127 may also function to facilitate alignment of the microfluidic cartridge 100 within an external molecular diagnostic module. In a first embodiment, as shown in FIG. 1B, the set of valve guides 127 comprises holes within the intermediate substrate 120 at the set of occlusion positions 141, with sloped edges configured to direct a pin or indenter through the holes. In the first embodiment, the set of valve guides 127 may be produced in the intermediate substrate 120 by injection molding, or may alternatively be produced by drilling, countersinking, chamfering, and/or beveling. In another embodiment, the set of valve guides 127 comprises grooves with holes, such that a pin or indenter is configured to travel along a groove and through a hole that defines the valve guide. In a simplified alternative variation, the set of valve guides 127 may comprise holes through the intermediate substrate 120, wherein the holes do not have sloped edges. In yet another simplified alternative variation, the set of valve guides 127 may comprise a slot configured to provide access to the elastomeric

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layer 140 by a group of occluding objects (e.g. pins or indenters), rather than a single occluding object.

1.3 Microfluidic Cartridge—Elastomeric and Bottom Layers

As shown in FIGS. 1B and 5A-5D, an embodiment of the microfluidic cartridge 100 also comprises an elastomeric layer 140 partially situated on the intermediate substrate 120, which functions to provide a deformable substrate that, upon deformation, occludes a microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 at an occlusion position of a set of occlusion positions 141. Preferably, the elastomeric layer 140 comprises an inert, liquid impermeable material, of an appropriate thickness, that can be heated to temperatures encountered during manufacturing and/or specified in a molecular diagnostic protocol, without substantial damage (i.e. compromised surface and/or loss of mechanical robustness) and is chemically compatible with a PCR assay. Preferably, the elastomeric layer 140 is non-continuous, such that portions of the elastomeric layer 140 are positioned relative to the intermediate substrate 120 in a manner that directly covers holes provided by the set of valve guides 127. Alternatively, the elastomeric layer 140 is a continuous layer, spanning a majority of the footprint of the microfluidic cartridge 100 while covering holes provided by the set of valve guides 127. In a specific embodiment, the elastomeric layer 140 comprises 500 micron thick strips of a low-durometer silicone that can be heated to at least 120° C. without substantial damage, which are bonded to a portion of the intermediate substrate 120 using a silicone-based adhesive and slightly compressed between the film layer 125 and the intermediate substrate 120. In a variation of the specific embodiment, the elastomeric layer 140 may alternatively be held in place solely by pressure between the intermediate layer 120 and the top layer 110. Preferably, the elastomeric layer 140 is reversibly deformable over the usage lifetime of the microfluidic cartridge 100, such that any occlusion of a microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 is reversible over the usage lifetime of the microfluidic cartridge. Alternatively, the elastomeric layer 140 may not be reversibly deformable, such that an occlusion of a microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 is not reversible.

The set of occlusion positions 141 preferably comprises at least two types of occlusion positions, as shown in FIG. 1C, including a normally open position 42 and a normally closed position 43. As shown in FIGS. 5A-5D, the elastomeric layer 140 at a normally open position 42 of the set of occlusion positions 141 may be closed upon occlusion by an occluding object (FIGS. 5B and 5D). Preferably, a normally open position 42 is configured to withstand pressures that can be generated by a fluid delivery system (e.g. a syringe pump) without leaking, upon occlusion by an occluding object at the normally open position 42. In one specific example, a 1/2 barrel-shaped pin head may be used to fully occlude a normally open position 42 having an arched cross section, as in FIG. 5C, with near constant pressure on the portion of the elastomeric layer compressed between the occluding object and occluding position.

The normally closed position 43 of the set of occlusion positions 141, functions to be normally closed, but to be forced open in response to fluid delivery by a fluid delivery system. In one variation, the normally closed position 43 may be formed by manufacturing (e.g. injection molding) the top layer 100, such that the top layer material at a normally closed position 43 extends down to the elastomeric layer 140. If an occluding object is held away from the normally closed position 43, the occlusion position is closed, but can be forced open due to fluid pressure applied by a fluid delivery system

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(e.g. syringe pump). When not in operation, however, the normally closed position 43 is configured to prevent leakage and/or fluid bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.

The microfluidic cartridge 100 may further comprise a bottom layer 170 configured to couple to the intermediate substrate, which functions to allow waste to be contained within the microfluidic cartridge 100, and allow microfluidic cartridges to be stacked. The bottom layer thus facilitates reception, isolation, and containment of a waste fluid within the waste chamber. Preferably, the bottom layer 170 is composed of the same material as the intermediate substrate 120 for cost and manufacturing considerations, and bonded to the intermediate substrate 120 in a manner that provides a hermetic seal, such that a liquid within the waste chamber 130 does not leak out of the waste chamber 130. In a specific embodiment, the bottom layer 170 and the intermediate substrate 120 are both composed of a polypropylene-based material, and bonded together using an adhesive. In an embodiment of the microfluidic cartridge 100 where the waste chamber 130 has a corrugated surface, the bottom layer 170 preferably only seals voids defining the waste chamber 130, such that non-waste chamber regions (i.e. non-waste housing regions) are not covered by the bottom layer 170. Alternatively, the microfluidic cartridge 100 may omit the bottom layer 170, such that any waste fluid that enters the waste chamber 130 completely leaves the microfluidic cartridge 100 and is collected off-cartridge by a waste-collecting subsystem of an external molecular diagnostic system. In this alternative embodiment, the intermediate substrate 120 is configured to fluidically couple to the waste-collecting subsystem.

1.4 Microfluidic Cartridge—Magnet Housing

The magnet housing region 150 of the microfluidic cartridge 100 functions to provide access to and/or house at least one magnet 152 providing a magnetic field 156 for purification and isolation of nucleic acids. Preferably, the magnet housing region 150 is defined by the film layer and the intermediate substrate, such that the film layer and the intermediate substrate form the boundaries of the magnet housing region 150. In an embodiment of the microfluidic cartridge 100 comprising a bottom layer 170, the magnet housing region 150 may further be defined by the bottom layer 170, such that the bottom layer partially forms a boundary of the magnet housing region 150. The magnet housing region 150 is preferably a rectangular prism-shaped void in the microfluidic cartridge 150, and accessible only through one side of the microfluidic cartridge 100, as shown in FIG. 1B. Preferably, the magnet housing region 150 can be reversibly passed over a magnet 152 to house the magnet 152, and retracted to remove the magnet 152 from the magnet housing region 150; however, the magnet 152 may alternatively be irreversibly fixed within the magnet housing region 150 once the magnet 152 enters the magnet housing region 150.

Preferably, the magnet housing region 150 is bounded on at least two sides by the waste chamber 130, and positioned near the middle of the microfluidic cartridge 100, such that a fluidic pathway 165 passing through the magnetic field 156 passes through the magnetic field 156 at least at one point along an intermediate portion of the fluidic pathway 165. Preferably, the magnet housing region 150 also substantially spans at least one dimension of the microfluidic cartridge, such that multiple fluidic pathways 165 of the microfluidic

cartridge 100 cross the same magnet housing region 150, magnet 152, and/or magnetic field 156. Alternatively, the magnet housing region 150 may be configured such that a magnet within the magnet housing region 150 provides a magnetic field spanning all fluidic pathways 165 of the microfluidic cartridge in their entirety. In alternative embodiments, the microfluidic cartridge may comprise more than one magnet housing region 150, a magnet housing region 150 may be configured to receive and/or house more than one magnet 152, and/or may not be positioned near the middle of the microfluidic cartridge 100. In yet another alternative embodiment, the magnet housing region 150 may permanently house a magnet 152, such that microfluidic cartridge comprises a magnet 152, integrated with the intermediate substrate 120. In embodiments where the magnet 152 is retractable from the microfluidic cartridge 100, the magnet 152 may be a permanent magnet or an electromagnet. In embodiments where the magnet 152 is configured to be integrated with the microfluidic cartridge 100, the magnet 152 is preferably a permanent magnet, which provides a stronger magnetic field per unit volume.

1.5 Microfluidic Cartridge—Fluidic Pathways

The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection. Preferably, each fluidic pathway 165 in the set of fluidic pathways 160 is formed by at least a portion of the top layer, a portion of the film layer, and a portion of the elastomeric layer 140, such that each fluidic pathway 165 may be occluded upon deformation of the elastomeric layer 140 at a set of occlusion positions 141. Additionally, at least one fluidic pathway 165 in the set of fluidic pathways 160 is preferably fluidically coupled to a sample port-reagent port pair 113 of the set of sample port-reagent port pairs 112, a fluid port 118, a waste chamber 130, and a detection chamber 117 of the set of detection chambers 116. Furthermore, at least one fluidic pathway 165 in the set of fluidic pathways 160 is preferably configured to be occluded upon deformation of the elastomeric layer 140, configured to transfer a waste fluid to the waste chamber 30, comprises a capture segment 166 passing through the heating region 195 and a magnetic field 156, and is configured to pass through the vent region 190 upstream of a detection chamber 117. Alternative embodiments may omit preferred elements of the embodiment of the fluidic pathway 165 described above, such as a vent region 190 or a heating region 195, or add additional elements to the embodiment of the fluidic pathway 165 described above.

A fluidic pathway 165 of the set of fluidic pathways 160 may comprise portions (i.e. microfluidic channels) that are located on both sides of the top layer 110, but is preferably located primarily on the bottom side of the top layer (in the orientation shown in FIG. 1B). In the orientation of the microfluidic cartridge 100 shown in FIG. 1B, a microfluidic channel on top of the top layer 110 may be further covered by second film layer 168 that seals the microfluidic channel on top of the top layer 110. The second film layer 168 may be comprise a cyclic olefin polymer (COP) film, thermally or adhesively bonded to the top layer 110, or alternatively may comprise another material that is bonded to the top layer 110. The use of film layers 125, 168 to cover microfluidic channels on either side of the top layer 11 facilitates manufacturing, such that long stretches of a fluidic pathway 165 do not need to be produced within the interior of the top layer 110. Pref-

erably, microfluidic channels may be etched, formed, molded, cut, or otherwise shaped into the rigid structure of the top layer 110, and either remain on one side of the top layer 110, or pass through the thickness of the top layer 110.

In one variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 11B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a vent region 190 on the top side of the top layer 110. All other segments of the fluidic pathway 165 are preferably located on the bottom side of the top layer 110, allowing the fluidic pathway 165 to be sealed by the film layer 125 without requiring a separate film layer to seal channels located on the top of the top layer 110.

In another variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 1B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a detection chamber 163 on the top side of the top layer 110 and a segment running away from the detection chamber 164 on the top side of the top layer 110. In this variation, the fluidic pathway 165 thus crosses the thickness of the top layer 110 upstream of the first segment running to the detection chamber 163, and crosses the thickness of the top layer 110 downstream of the segment running away from the detection chamber 164, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110. In another variation, as shown in FIG. 6C, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising only a segment running away from the detection chamber 164 on the top side of the top layer 110. In this other variation, the fluidic pathway 165 thus crosses the thickness of the top layer 110 downstream of the second portion, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110. Alternatively, other embodiments may comprise a fluidic pathway 165 with a different configuration of portions on the top side of the top layer 110 and/or portions on the bottom side of the top layer 110.

As shown in FIGS. 1C, 6C, 7 and 9, a fluidic pathway 165 of the set of fluidic pathways 160 is branched and preferably comprises an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a fluid port 118, a sample segment 175 coupled to a sample port 114, a reagent segment 176 coupled to a reagent port 115, a capture segment 166 passing through at least one of the heating region 195 and a magnetic field 156, a vent segment 177 configured to pass through the vent region 190, a segment running to a detection chamber 163, a segment running away from the detection chamber 164, and at least one waste segment 178, 179 configured to transfer a waste fluid to a waste chamber 130. Individual segments of the fluidic pathway 165 are preferably configured to pass through at least one occlusion position of the set of occlusion positions 141, to controllably direct fluid flow through portions of the fluidic pathway 165. A fluidic pathway 165 may also further comprise an end vent 199, which functions to prevent any fluid from escaping the microfluidic channel.

The initial segment 174 of the fluidic pathway 165 functions to deliver common liquids and/or gases from a fluid port 118 through at least a portion of the fluidic pathway 165, the sample segment 175 functions to deliver a volume of a sample fluid (e.g. sample comprising nucleic acids bound to magnetic beads) to a portion of the fluidic pathway 165, and the reagent segment 176 functions to deliver a volume of fluid comprising a reagent to a portion of the fluidic pathway 165. The capture segment 166 functions to facilitate isolation and purification of nucleic acids from the volume of the sample fluid, and may be s-shaped and/or progressively narrowing, to

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increase the efficiency and/or effectiveness of isolation and purification. Alternatively, the capture segment **166** may altogether be replaced by a substantially straight portion **166** or any other geometric shape or configuration that functions to facilitate isolation and purification of nucleic acids from the volume of the sample fluid. The capture segment **166** of the fluidic pathway **165** preferably has an aspect ratio less than one, which functions to facilitate capture of magnetic particles, but may alternatively have an aspect ratio that is not less than one.

The vent segment **177** functions to deliver a processed sample fluid through the vent region **190** for gas removal. The segment running to a detection chamber **163** functions to deliver a processed sample fluid to the detection chamber **117** with a reduced quantity of gas bubbles, and the segment running away from the detection chamber **164** functions to deliver a fluid away from the detection chamber **117**. The segments may be arranged in at least one of several configurations to facilitate isolation, processing, and amplification of a nucleic acid sample, as described in three exemplary embodiments below:

A first embodiment, as shown in FIG. **1C**, of a fluidic pathway **165** preferably comprises an initial segment **174** fluidically coupled to a fluid channel **119** coupled to a shared fluid port **118**, a sample segment **175** coupled to a sample port **114** and to the initial segment **174**, and an s-shaped capture segment **166**, configured to pass through the heating region **195** and a magnetic field **156**, coupled to the initial segment **174** and the sample segment **175**. In a variation of the first embodiment, the s-shaped capture segment **166** may comprise an initial wide arc **166** to provide a greater surface area for magnetic bead capture. In another variation of the first embodiment, the capture segment **166** may alternatively be a progressively narrowing s-shaped capture segment **166**. The first embodiment of the fluidic pathway **165** also comprises a reagent segment **176** coupled to a reagent port **115** and to the capture segment **166**, a vent segment **177** coupled to the reagent segment **176** and configured to pass through the vent region **190**, a segment running to a detection chamber **163** from the vent region **190**, a winding segment running away from the detection chamber **164**, and an end vent **199** coupled to the segment running away from the detection chamber **164**. The first embodiment of the fluidic pathway **165** also comprises a first waste segment **178** configured to couple the initial segment **174** to the waste chamber **130**, and a second waste segment **179** configured to couple the capture segment **166** to the waste chamber **130**. The first waste segment **178** preferably functions to allow evacuation of excess release fluids from a fluidic pathway **165**, for precise metering of the amount of release reagents used in a molecular diagnostic procedure using a low volume of sample.

In the first embodiment, the set of occlusion positions **141** comprises a first occlusion position **142** located along the initial segment **174** between points at which the initial segment couples to the fluid channel **119** and to the capture segment **166**. The set of occlusion positions **141** also comprises a second occlusion position **143** located along the sample segment **175**, a third occlusion position **144** located along the reagent segment **176**, a fourth occlusion position **145** located along the first waste segment **178**, and a fifth occlusion position **146** located along the second waste segment **179**. In the first embodiment, the set of occlusion positions **141** also comprises a sixth occlusion position **147** located along the vent segment **177** upstream of the vent region **190**, a seventh occlusion position **148** located along the segment running to the detection chamber **163**, and an eighth occlusion position **149** located along the segment running

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away from the detection chamber **164**. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions **142**, **143**, **144**, **146**, **147** are normally open positions **42** and the fourth, seventh, and eighth occlusions positions **145**, **148**, **149** are normally closed positions **43**, as shown in FIG. **1C**.

The occlusion positions of the set of occlusion positions **141** of the first embodiment are preferably located such that occluding subsets of the set of occlusion positions **141** defines unique truncated fluidic pathways to controllably direct fluid flow. For example, as shown in FIG. **1D**, occluding the fluidic pathway **165** at the first, third, fourth, and sixth occlusion positions **142**, **144**, **145**, **147** forms a truncated pathway by which a volume of a sample fluid, comprising nucleic acids bound to magnetic beads and delivered into the sample port **114**, may flow past the second occlusion positions **143** into the capture segment **166** for isolation and purification of nucleic acids using the heating region **195** and the magnetic field **156**. Nucleic acids bound to magnetic beads may thus be trapped within the capture segment **166** by the magnetic field **156**, while other substances in the volume of sample fluid may pass into the waste chamber **130** by passing the fifth occlusion position **146**. Following this subset of occlusion positions, the occlusion at the first occlusion position **142** may be reversed, as shown in FIG. **1E**, and the fluidic pathway **165** may be occluded at the second occlusion position **143** to form a second truncated pathway by which a wash fluid may be delivered through the fluid port **118**, into the capture segment **166** (thus washing the trapped magnetic beads), and into the waste chamber **130** by passing the fifth occlusion position **146**. The occlusion at the second occlusion position **143** may then be reversed, and the first occlusion position **142** may be occluded (as shown in FIG. **1D**), so that other fluidic pathways in the set of fluidic pathways **160** may be washed. After all fluidic pathways have been washed, a volume of air may be transferred through the fluid port **118** to prevent mixture of a wash solution with a release solution.

Thereafter in the first embodiment, as shown in FIG. **1E**, the fluidic pathway **165** may be occluded at the second occlusion position **143** and the occlusion at the first occlusion **142** may be reversed, thus creating a third truncated pathway as shown in FIG. **1D**. A release solution may then be delivered through the fluid port **118**, into the capture segment **166**, and to the waste chamber **130** by passing the fifth occlusion position **146**. The release solution may then be sealed within a fourth truncated pathway (including the capture segment **166**) of the fluidic pathway **165** by occluding the fluidic pathway at the fifth occlusion position **146**, as shown in FIG. **1F**. A release solution may then be delivered to other fluidic pathways of the set of fluidic pathways **160**.

Thereafter, as shown in FIG. **1G**, the occlusion at the fourth occlusion position **145** may be reversed, creating a fifth truncated pathway, and release solution within the fluidic pathway **165** may be metered by pumping air through the fluid port **118**, which functions to push a portion of the release solution into the waste chamber **130**. A volume of release solution will still be maintained within the capture segment **166** at this stage. As shown in FIG. **1H**, the first and the fourth occlusion positions **142**, **145** may then be occluded to form a sixth truncated pathway sealing the volume of release solution, with the captured magnetic beads bound to nucleic acids, within the capture segment **166**. The volume of the remaining release solution is therefore substantially defined by the microchannel volume between junctions in the fluidic pathway **165** near the fourth and sixth occlusion positions **145**, **147**, and may be any small volume but in a specific variation is precisely metered to be 23+/-1 microliters.

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Release solution may be sealed within capture segments of other fluidic pathways using a similar process. A heater may then be provided at the sixth truncated pathway, inducing a pH shift within the sixth truncated pathway to unbind nucleic acids from the magnetic beads.

Thereafter in the first embodiment, as shown in FIG. 1I, the occlusions at the first and third occlusion positions **142**, **144** may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. ~20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port **115**. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge **100**. During the reconstitution, the occlusion at the sixth occlusion position **147** may be reversed, and the fluidic pathway **165** may be occluded at the first occlusion position **142** to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port **115**, through the eighth truncated pathway, and to the detection chamber **117**, by using a fluid handling system to push the seventh occlusion position (normally closed) open. The detection chamber **117** is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway **165** is occluded at the third, sixth, seventh and eighth occlusion positions **144**, **147**, **148**, **149**, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways **165** may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber **117**.

An alternative variation of the first embodiment may further comprise additional occlusion positions or alternative variations of the set of occlusion positions **141**, such that occlusion at the additional occlusion positions permanently seals the waste chamber from the fluidic pathway **165**. Other alternative variations of the first embodiment may also comprise configurations of the set of occlusion positions **141** that are different than that described above. The variations may be configured, such that the a fluidic pathway **165** facilitates meter release, does not allow meter release, facilitates addition of other reagents (e.g. neutralization or DNase reagents), facilitates additional washing steps, and/or facilitates other operations without changing the layout of the fluidic pathway **165** of a microfluidic cartridge embodiment. Thus, multiple unique operations may be performed using the same microfluidic cartridge, by occluding fluidic pathways **160** at varied subsets of a set of occlusion positions **141**.

A second embodiment, as shown in FIG. 6C, of a fluidic pathway **165'** preferably comprises an initial segment **174'** fluidically coupled to a fluid channel **119'** coupled to a shared fluid port **118'**, a sample segment **175'** coupled to a sample port **114'** and to the initial segment **174'**, and a capture segment **166'**, configured to pass through the heating region **195** and a magnetic field **156**, coupled to the initial segment **174'**. The second embodiment of the fluidic pathway **165'** also comprises a reagent segment **176'** coupled to a reagent port **115'** and to the turnabout portion **176'**, a vent segment **177'** coupled to the reagent segment **176'** and to the capture segment **166'** and configured to pass through the vent region **190**, a segment running to a detection chamber **163'** from the vent region **190**, a segment running away from the detection chamber **164'**, and an end vent **199** coupled to the segment running away from the detection chamber **164'**. The second embodiment of the fluidic pathway **165'** also comprises a first waste

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segment **178'**, coupled to the initial segment **174'** at a point between points connecting the initial segment **174'** to the sample segment **175'** and to the capture segment **166'**. The first waste segment **178'** is configured to couple the initial segment **174'** to the waste chamber **130**. The second embodiment of the fluidic pathway **165'** also comprises a second waste segment **179'** configured to couple the capture segment **166'** to the waste chamber **130'**, and an end vent segment **197'** coupled to the capture segment **166'** downstream of the point of connection to the second waste segment **179'**, and coupled to an end vent **199**. The end vent segment **197'** functions to provide fine metering of a fluid flowing through the fluidic pathway **165'**.

In the second embodiment, the set of occlusion positions **141'** comprises a first occlusion position **142'** located along the initial segment **174'** between points at which the initial segment couples to the fluid channel **119'** and to the sample segment **175'**. The set of occlusion positions **141'** also comprises a second occlusion position **143'** located along the sample segment **175'**, a third occlusion position **144'** located along the reagent segment **176'**, a fourth occlusion position **145'** located along the first waste segment **178'**, and a fifth occlusion position **146'** located along the second waste segment **179'**. In the second embodiment, the set of occlusion positions **141'** also comprises a sixth occlusion position **147'** located along the vent segment **177'** upstream of the vent region **190**, a seventh occlusion position **148'** located along the segment running to the detection chamber **163'**, and an eighth occlusion position **149'** located along the segment running away from the detection chamber **164'**. Additionally, in the second embodiment, the set of occlusion positions **141** comprises a ninth occlusion position **157'** located along the sample segment **175'** between the sample port **114** and the second occlusion position **143**, a tenth occlusion position **158'** located along the end vent segment **197'**, and an eleventh occlusion position **159'** located along the capture segment **166'** between points at which the capture segment **166'** couples to the end vent segment **197'** and to the vent segment **177'**.

The occlusion positions of the set of occlusion positions **141'** of the second embodiment are preferably located such that occluding of subsets of the set of occlusion positions **141'** defines unique truncated fluidic pathways to controllably direct fluid flow. For example, occluding the fluidic pathway **165'** at the first, fourth, sixth, tenth, and eleventh occlusion positions **142'**, **145'**, **147'**, **158'**, **159'** forms a truncated pathway by which a volume of a sample fluid, comprising nucleic acids bound to magnetic beads and delivered into the sample port **114**, may flow into the capture segment **166'** for isolation and purification of nucleic acids using the heating region **195** and the magnetic field **156**. Nucleic acids bound to magnetic beads may thus be trapped within the capture segment **166'** by the magnetic field **156**, while other substances in the volume of sample fluid may pass into the waste chamber **130** by passing the fifth occlusion position **146'**. Following this subset of occlusion positions, the occlusion at the first occlusion position **142'** may be reversed, and the fluidic pathway **165'** may be occluded at the second occlusion position **143'** to form a second truncated pathway by which a wash fluid may be delivered through the fluid port **118**, into the capture segment **166'** (thus washing the trapped magnetic beads), and into the waste chamber **130** by passing the fifth occlusion position **146'**. A volume of air may then be pumped through the fluid port **118** to flush any remaining wash solution into the waste chamber **130**.

Thereafter, in the second embodiment, the fluidic pathway **165'** may be occluded at the fifth occlusion position **146'** and

the occlusion at the tenth occlusion position **158'** may be reversed, closing access to the waste chamber **130** and opening access to the end vent segment **197'**. A release solution may then be delivered through the fluid port **118**, into the capture segment **166'**, and to the end vent segment **197'**. The volume of the release solution is therefore defined by the microchannel volume between the fourth and tenth occlusion positions **145'**, **158'**, and may be any small volume but in a specific variation is precisely metered to be 15 microliters. Thereafter, occluding the fluidic pathway **165'** at the tenth occlusion position **158'**, reversing the occlusion at the fourth occlusion position **145'** (defining a fourth truncated pathway), and delivering air through the fluid port **118** pushes any remaining release buffer from the fluidic pathway **118** into the waste chamber **130**, thereby ensuring that excess release buffer is not later exposed to nucleic acids bound to the magnetic beads (at this point, the nucleic acids are not substantially released from the magnetic beads because heat has not been added). Thereafter, the fluidic pathway **165'** is occluded at the first and fourth occlusion positions **142'**, **145'**, defining a fifth truncated pathway comprising the capture segment **166'**, and the magnetic beads are heated to an appropriate temperature and time (e.g., 60 degrees for 5 minutes) within the heating region **195** to release the nucleic acids from the magnetic beads and into the release buffer.

Thereafter, in the second embodiment, the occlusions at the first and eleventh occlusion positions **142'**, **159'** are reversed, defining a sixth truncated pathway, the entire released nucleic acid sample (e.g. ~15 microliters) may be aspirated out of the microfluidic cartridge through the reagent port **115**. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent mixture stored off of the microfluidic cartridge **100**. During the reconstitution process, the occlusion at the sixth occlusion position **147'** may be reversed, thus defining a seventh truncated pathway. Once reconstitution of the molecular diagnostic reagent mixture with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be aspirated through the reagent port **115** through the seventh truncated pathway to the detection chamber **117**, completely filling the detection chamber **117**, after which the fluidic pathway **165'** is be occluded at third, seventh, eighth, and ninth occlusion positions **144'**, **148'**, **149'**, **157'** defining an eighth truncated pathway. An external molecular diagnostic system and/or module may then perform additional processes on the volume of fluid within the detection chamber **117**.

An alternative variation of the second embodiment may further comprise additional occlusion positions or alternative variations of the set of occlusion positions **141'**, such that occlusion at the additional occlusion positions permanently seals the waste chamber from the fluidic pathway **165'**. Other alternative variations of the second embodiment may also comprise configurations of the set of occlusion positions **141'** that are different than that described above.

A third embodiment, as shown in FIG. 7, of a fluidic pathway **165"** preferably comprises an initial segment **174"** fluidically coupled to a fluid channel **119"** coupled to a shared fluid port **118**, a sample segment **175"** coupled to a sample port **114** and to the initial segment **174"**, and a capture segment **166"** coupled to the initial segment **174"**. The third embodiment of the fluidic pathway **165"** also comprises a reagent segment **176"** coupled to a reagent port **115**, a vent segment **177"** coupled to the reagent segment **176"** and to the capture segment **166"**, and configured to pass through the vent region **190**, a segment running to a detection chamber **163"** from the vent region **190**, a segment running away from the detection chamber **164"**, and an end vent **199** coupled to

the segment running away from the detection chamber **164"**. The third embodiment of the fluidic pathway **165"** also comprises a first waste segment **178"** configured to couple the initial segment **174"** to the waste chamber **130**, and a second waste segment **179"** configured to couple the capture segment **166"** to the waste chamber **130**.

In the third embodiment, the set of occlusion positions **141"** comprises a first occlusion position **142"** located along the initial segment **174"** between points at which the initial segment **174"** couples to the fluid channel **119"** and to the sample segment **175"**. The set of occlusion positions **141"** also comprises a second occlusion position **143"** located along the sample segment **175"**, a third occlusion position **144"** located along the reagent segment **176"**, a fourth occlusion position **145"** located along the first waste segment **178"**, and a fifth occlusion position **146"** located along the second waste segment **179"**. In the third embodiment, the set of occlusion positions **141"** also comprises a sixth occlusion position **147"** located along the vent segment **177"** upstream of the vent region **190**, a seventh occlusion position **148"** located along the segment running to the detection chamber **163"**, an eighth occlusion position **149"** located along the segment running away from the detection chamber **164"**, and a ninth occlusion position **157"** located along the vent segment **177"** between the point at which the vent segment **177"** couples to the second waste segment **179"** and the sixth occlusion point **147"**.

Similar to the first and the second embodiments, the occlusion positions of the set of occlusion positions **141"** of the third embodiment are preferably located such that an occlusion of subsets of the set of occlusion positions **141"** defines unique truncated fluidic pathways to controllably direct fluid flow. Example truncated fluidic pathways, defined by occluding the fluidic pathway **165"** using subsets of the set of occlusion positions **141"**, are shown in FIG. 7.

Preferably, a fluidic pathway **165** of the set of fluidic pathways **160** comprises at least one of a first channel type **171**, a second channel type **172** with a reduced cross sectional area, and a third channel type **173** with a curved surface as shown in FIG. 8A. A variation of the first channel type **171** has an approximately rectangular cross section with slightly sloping walls, such that at least two walls of the first channel type **171** slope toward each other to facilitate manufacturing of the first channel type **171**; however, alternative variations of the first channel type **171** may have non-sloping walls or walls that slope away from each other. In specific embodiments of the first channel type **171**, the walls of the first channel type **171** slope at 6° from vertical, to facilitate extraction of injection molded parts, and are between 300 and 1600 microns wide and between 100 and 475 microns tall. In a first specific embodiment of the second channel type **172**, the cross section of the second channel type **172** is a 250 micron wide equilateral triangle with the top truncated to be 200 microns deep. In a second specific embodiment of the second channel type **172**, the cross section of the second channel type is a truncated triangle that is 160 microns wide and 160 microns deep. In a specific embodiment of the third channel type **173**, the surface of the third channel type is defined by Gaussian function, and is 800 microns wide and 320 microns deep. Alternative embodiments of the third channel type **173** may comprise a surface defined by any appropriate curved function.

The first channel type **171** is preferably used over a majority of a fluidic pathway **165**, and preferably in portions near a vent region **190**, in a capture segment **166** configured to pass through a magnetic field **156**, and in a segment leading to a Detection chamber **163**. Preferably, an embodiment of the first channel type **171**, comprising a wide channel with little

depth is used in regions configured to pass through a magnetic field **156**, such that particles in the regions are driven closer to the magnetic field source. The second channel type **172** is preferably used near a vent region **190** of a fluidic pathway **165**, and preferably in portions of a fluidic pathway **165** leading to and away from a detection chamber **163**, **164** (to constrict fluid flow into the Detection chamber **117**). The third channel type **173** is preferably used in a portion of a fluidic pathway **165** near a normally open position **42** of the set of occlusion positions **141**. Transitions between different channel types **171**, **172**, **173** may be abrupt, or alternatively, may be gradual, as shown in FIG. **8B**. The first, second, and third channel types **171**, **172**, **173** may also alternatively be used in any appropriate portion of a fluidic pathway **165**. Example embodiments of channel types for segments of a fluidic pathway are shown in FIG. **8C**.

Multiple fluidic pathways may be configured to pass through a single heating region **195** of the microfluidic cartridge **100**, a single vent region **190** of the microfluidic cartridge **100**, and/or a magnetic field **156** produced by a magnet **152** housed within a single magnet housing region **150**. Preferably all fluidic pathways of the set of fluidic pathways **160** are configured to pass through a single heating region **195** of the microfluidic cartridge **100**, a single vent region **190** of the microfluidic cartridge **100**, and a magnetic field **156** produced by a magnet **152** housed within a single magnet housing region **150**; however, alternative embodiments of the set of fluidic pathways **160** of the microfluidic cartridge may comprise different configurations wherein fluidic pathways of the set of fluidic pathways **160** do not share a single heating region **195**, a single vent region **190**, and/or a magnetic field **156**.

Additionally, the set of fluidic pathways **160** of the microfluidic cartridge **100** may comprise virtually any number of fluidic pathway **165** and/or the set of Detection chambers **116** may comprise virtually any number of Detection chambers **116** as can practically be integrated into the microfluidic cartridge **100**. In one specific embodiment, the set of fluidic pathways **160** may comprise twelve fluidic pathways **165**, four of which are shown in FIG. **9**.

1.6 Microfluidic Cartridge—Additional Microfluidic Cartridge Elements

The microfluidic cartridge **100** is preferably configured such that actual valving members are not integrated into the microfluidic cartridge **100**, thus, opening and/or occluding portions of a fluidic pathway **165** are performed by systems located external to the microfluidic cartridge. As an example, portions of a fluidic pathway **165** may be opened or occluded at occlusion positions, as described above, by the action of a valving member or mechanism held beneath the card that applies a biasing force to deform the elastomeric layer **140** and occlude a fluidic pathway **165**. The force may be applied by a mechanical member (e.g., a pin, post, etc.), an electro-mechanical member (e.g. a solenoid), a pneumatic or hydraulic member (e.g., air, water, etc.) or any other appropriate means, as shown in FIGS. **10A** and **10B**. In some variations, the cartridge may include one or more registration regions that allow the card to be aligned with respect to the valving member or mechanism. In alternative embodiments, the elastomeric layer **140**, the set of valve guides **127**, and the set of occlusion positions **141** may be omitted and replaced with valves integrated within the microfluidic cartridge **100**, that are configured to controllably occlude and open portions of a fluidic pathway **165**.

Other embodiments of the microfluidic cartridge **100** may further comprise a tag **198** that functions to encode and provide identifying information related to the microfluidic car-

tridge **100**. The tag **198** may comprise a barcode, QR code, or other optical machine-readable tag, or may alternatively be an electronic tag, such as an RFID chip. The identifying information preferably comprises at least information relating to the position of a microfluidic cartridge **100** within a molecular diagnostic system, and information relating to samples analyzed using the microfluidic cartridge **100** (e.g. how many positions remain available for conducting tests). In alternative variations, the tag may relate other information about samples (e.g. sample type, sample volume, sample concentration, date) processed using the microfluidic cartridge **100**. Preferably, the tag does not interfere with procedures being performed using the microfluidic cartridge, and is located in an unobtrusive position on the microfluidic cartridge **100**, such as a side panel of the microfluidic cartridge **100**. Alternatively, the microfluidic cartridge **100** may not comprise a tag **198**, and a user or other entity may relate identifying information to the microfluidic cartridge **100** using any appropriate element.

As a person skilled in the art will recognize from the previous detailed description and from the FIGURES and claims, modifications and changes can be made to the preferred embodiments of the microfluidic cartridge **100** without departing from the scope of this invention, as is shown in the example embodiment shown in FIGS. **11A** and **11B**, and in the alternative example embodiment of FIGS. **6A-6C**, wherein in the orientation of FIG. **6B**, the intermediate substrate **120** comprising a waste chamber **130** is coupled to the top layer **110**, and the elastomeric layer **140** is located on the bottom of the microfluidic cartridge **100**.

2. Specific Embodiment of a Microfluidic Cartridge

The following description a specific embodiment of the microfluidic cartridge **100** is for illustrative purposes only, and should not be construed as definitive or limiting of the scope of the claimed invention.

The specific embodiment of the microfluidic cartridge **100**, as shown in FIGS. **11A** and **11B**, meets SLAS ANSI guidelines for a microtiter plate footprint, governing the dimensions of the specific embodiment of the microfluidic cartridge **100**. The specific embodiment of the microfluidic cartridge **100** is thus 127.76 mm long and 85.48 mm wide.

The specific embodiment of the microfluidic cartridge **100** comprises a top layer **110** including a set of twelve sample port-reagent port pairs **112**, a set of twelve Detection chambers **116**, a shared fluid port **118**, a heating region **195**, and a vent region **190**, an intermediate substrate **120**, coupled to the top layer **110** and partially separated from the top layer **110** by a film layer **125**, configured to form a waste chamber **130**, an elastomeric layer **140** partially situated on the intermediate substrate **120**; a magnet housing region **150** accessible by a magnet **152** providing a magnetic field **156**; a bottom layer **170** coupled to the intermediate substrate **120** and configured to seal the waste chamber, and a set of fluidic pathways **160**, formed by at least a portion of the top layer **110**, a portion of the film layer **125**, and a portion of the elastomeric layer **140**.

The top layer **110** of the specific embodiment of the microfluidic cartridge **100** functions preferably as described in Section 1.1, and is composed of polypropylene with low autofluorescence and a glass transition temperature suitable for PCR. The majority of the top layer **110** of the specific embodiment is 1.5 mm thick (aside from regions defining ports, the vent, the heating region **195** or fluidic pathways **165**), and is produced by injection molding without the use of a mold release. The polypropylene is clear to allow transmission of light in the detection chambers. The injection molding process defines the set of 12 sample port-reagent port pairs, which are located along one long edge of the top layer **110**,

and also defines the set of 12 detection chambers **116**, which are located along the opposite long edge of the top layer **110**. The Detection chambers **117** do not completely transect the top layer **110**, as shown in FIGS. **11A** and **11B**. Each detection chamber **117** of the specific embodiment is identical and comprised of three interconnected channels, configured in a circular arrangement, with each of the interconnected channels approximately 0.4 mm deep and 1.6 mm wide at its widest point, resulting in a total volume of ~10 mL for each detection chamber **117**. The dimensions of the detection chambers **117** of the specific embodiment are such that the detection chambers **117** facilitate heating from one side (resulting in simpler heater design yet fast cycling given the small depth of the channels), and also facilitate the injection molding process. The bottoms of the detection chambers **117** are formed by the film layer **125**, which is polypropylene film compatible with PCR (100 microns thick or less) that offers low autofluorescence. The film layer **125** can withstand temperatures up to 120° C. or more.

The injection molding process also defines the shared fluid port **118** of the top layer **110**, and the vent region **190**, which is recessed 0.5 mm into the top surface of the top layer **110** (in the orientation shown in FIG. **11B**), and is covered with a polytetrafluoroethylene membrane, which is hydrophobic, gas permeable, and liquid impermeable. A paper label is bonded with adhesive to the top layer **110** over the vent region **190**, which serves to identify the cartridge and protect the vent region **190**, as shown in FIGS. **11A** and **11B**. The injection molding process also defines the heating region **195**, which is recessed and spans the long dimension of the top layer **110**, slightly offset from a midline of the top layer **110**. The top layer **110** of the specific embodiment requires approximately 15 grams of polypropylene, and all draft angles for the top layer **110** are a minimum of 4 degrees, as defined by the injection molding process.

In the specific embodiment, the intermediate substrate **120** is composed of a polypropylene material to minimize cost and simplify assembly, and in the orientation shown in FIG. **11B**, the top of the intermediate substrate **120** is 1.5 mm thick. The film layer **125**, partially separating the intermediate substrate **120** from the top layer **110** is a polypropylene film with a nominal thickness of 50 microns. The film layer **125** is able to withstand temperatures of up to 95° C. encountered during fabrication and during an intended PCR procedure, while being thermally bondable to the top layer **110**. The top layer **110** and the film layer **125** are bonded using thermal fusion bonding, and this subassembly is bonded to the intermediate substrate **120** using a polymer adhesive. Additionally, for aligning layers **110**, **120**, **125** and bonding the top layer **110** to the intermediate substrate **120**, plastic studs are configured to extend from the top of the intermediate substrate **120** through die-cut holes in the film layer **125** and injection molded holes in the bottom of the top layer **110**. The intermediate substrate also comprises a set of valve guides **127**, at a set of occlusion positions **141**, which are holes with chamfered edges through the intermediate substrate **127**. Each valve guide in the set of valve guides **127** is 2.1 mm×2.1 mm square, and configured to accommodate an occluder with a 2 mm×2 mm square head for normally open positions **42** or 2.1 mm diameter circle to accommodate a 2 mm diameter round pin for normally closed positions **43**.

The elastomeric layer **140** of the specific embodiment is composed of a low durometer silicone, and comprises strips that are 500 microns thick and that can withstand temperatures of 120° C. at a minimum. The strips of the elastomeric layer are arranged over the set of valve guides **127**, and bonded to the top of the intermediate substrate **120** using a

silicone adhesive. Additionally, the elastomeric layer **140** is slightly compressed between the film layer **125** and the top of the intermediate substrate (in the orientation shown in FIG. **11B**).

The bottom layer **170** of the specific embodiment of the microfluidic cartridge **100** is composed of polypropylene, identical to that of the intermediate substrate **120**. The bottom layer is 1.5 mm thick, and is contiguous in the area of the set of Detection chambers **116**, such that an outer perimeter of the entire bottom layer **170** substantially spans the footprint of the microfluidic cartridge **100**. The bottom layer **170** of the specific embodiment is bonded to the intermediate substrate **120** using polymer adhesive, providing a hermetic seal that ensures that a waste fluid within the waste chamber **130** of the intermediate substrate **120** does not leak out of the waste chamber **130**.

The specific embodiment of the microfluidic cartridge **100** comprises twelve fluidic pathways **165** in the set of fluidic pathways **160**, such that the microfluidic cartridge **100** is capable of testing up to twelve samples using twelve distinct fluidic pathways **165**. Each of the twelve fluidic pathways **165** is coupled to one of the twelve sample port-reagent port pairs **113** on one end of the microfluidic cartridge **100**, and coupled to one of the twelve detection chambers **117** on the other end of the microfluidic cartridge, as shown in FIGS. **11A** and **11B**. Each fluidic pathway **165** is substantially identical (aside from portions connecting to an initial segment **174** fluidically coupled to a fluid channel **119** coupled to a fluid port **118**) and identical to the first embodiment of a fluidic pathway described in Section 1.5 and shown in FIG. **1C**. Additionally, the microfluidic channels comprising each fluidic pathway **165** are of the first channel type **171** and 500 microns wide by 475 microns deep, aside from the microfluidic channels of the segments leading to and away from the detection chambers **163**, **164**, the turnabout portions **166**, and the vent segments **177**. Also, parallel microfluidic channels of the fluidic pathways **165** of the specific embodiment are typically evenly spaced at 2.25 mm (center-to-center).

The fluidic pathways **165** of the specific embodiment are, in their default condition, open at all occlusion positions, aside from the fourth, seventh, and eighth, occlusion positions **145**, **148**, **149**, as shown in FIG. **1C**. Furthermore, the s-shaped capture segment **166** of a fluidic pathway of the specific embodiment is configured to have a volume capacity of 22 µL, have a width of 5.5 mm, and weave back and forth over a magnetic field **156**, by crossing the magnet housing region **150**. The depth of the s-shaped capture segment **166** is 0.4 mm for the 1.6 mm wide channels and 0.475 for the 0.5 mm narrower channel.

The specific embodiment also comprises a barcode tag **198** located on a vertical edge of the microfluidic cartridge **100**, as shown in FIG. **11A**. Additional features of the specific embodiment of the microfluidic cartridge **100** are shown in FIGS. **11A** and **11B**.

3. Assembly Method for an Embodiment of the Microfluidic

An embodiment of an assembly method **200** for an embodiment of the microfluidic cartridge **100** is shown in FIGS. **12A-12G**. The assembly method **200** preferably comprises aligning the top layer to the film layer and thermally bonding the two, using silicone adhesive to bond the elastomeric layer to the intermediate substrate of the microfluidic cartridge **S210**, compressing the top layer, the film layer, the elastomeric layer, and the intermediate substrate and bonding the top/film layers to the elastomeric layer/intermediate substrate **S220**, bonding the intermediate substrate to the bottom layer **S230**, installing the vents of the vent region **S250**, and applying labels and packaging **S260**.

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Step S210 recites aligning the top layer to the film layer and thermally bonding the two, using silicone adhesive to bond the elastomeric layer to the intermediate substrate of the microfluidic cartridge, and functions to create a first subassembly comprising the top layer, the film layer, the elastomeric layer, and the intermediate substrate. Preferably, the elastomeric layer is glued with silicone to the intermediate substrate; however, the elastomeric layer may alternatively be solely compressed between the top layer/film layer and the intermediate substrate, without any adhesive. Preferably, a first jig is used to align the top layer and the film layer using pins in the jig and holes in the layers, and in an example embodiment of S210, the top layer is first placed face down in the first jig, and the film layer is placed onto the top layer in preparation for thermal bonding using a lamination machine or hot press. In the example embodiment of S210, the elastomeric layer is then fit over ultrasonic welding tabs in of the top layer, as shown in FIGS. 12D and 12F, however, processes other than ultrasonic welding may be used. An adhesive may also be applied around the border of the elastomeric layer, to prevent leakage between the elastomeric layer and the intermediate substrate. Protrusions molded into the top of the intermediate substrate are then passed through alignment holes in the top layer, thus aligning the top layer, the elastomeric layer, and the intermediate substrate of the microfluidic cartridge. In alternative embodiments of S210, any appropriate alignment mechanism may be used to align the top layer, the elastomeric layer, and the intermediate substrate, using for example, a combination of adhesives, frames, and alignment pins/recesses.

Step S220 recites compressing the top layer, the film layer, the elastomeric layer, and the intermediate substrate and bonding the top/film layers to the elastomeric layer/intermediate substrate, and functions to seal the layers in order to prevent leakage between the layers. Preferably, S220 forms hermetic seals between the top layer and the elastomeric layer, and the elastomeric layer and the intermediate substrate, in embodiments of S210 where an adhesive application is involved. In an example embodiment of S220, the first jig with the top layer, the elastomeric layer, and the intermediate substrate is placed within an ultrasonic welder to be compressed and ultrasonically welded.

Step S230 recites bonding the intermediate substrate to the bottom layer S230, which functions to form a second subassembly comprising the top layer, the elastomeric layer, the intermediate substrate, and the bottom layer. Preferably, the bottom layer self-aligns with the intermediate substrate as a result of the bottom layer fitting completely inside a recessed flange on the lower portion of the intermediate layer. The bottom layer is preferably thermally bonded to the intermediate layer. Alternatively, the bottom layer may be bonded to the intermediate layer using adhesive or ultrasonic welding, as shown in FIG. 12G.

Step S250 recites installing the vents of the vent region S250, which functions to permanently form the vents of the vent region. Step S250 is preferably performed by heat staking the vents in place, but may alternatively be performed using adhesive or solvent bonding process. Following step S250, the assembly method 200 may further comprise certain quality control measures, including pressure testing the microfluidic cartridge S252 by blocking all sample and reagent ports, and injecting air into the fluid port, and removing the finished microfluidic cartridge from the second jig S254. Step S260 recites applying labels and packaging, and functions to prepare the microfluidic cartridge with identifying information using at least a barcode label, and preparing the microfluidic cartridge for commercial sale.

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An alternative embodiment of an assembly method 300, as shown in FIG. 13, comprises thermally bonding the film layer to the top layer to form a first subassembly S310; adding a vent to the first subassembly and applying a label to create a second subassembly S320; applying an adhesive inside a bottom flange of the intermediate substrate and bonding the bottom layer to the intermediate substrate S330; applying a tag to the intermediate substrate to create a third subassembly S340; positioning the elastomeric layer on the third subassembly to create a fourth subassembly S350; applying adhesive to the fourth subassembly S360; and coupling the second subassembly to the fourth subassembly S370.

The FIGURES illustrate the architecture, functionality and operation of possible implementations of methods according to preferred embodiments, example configurations, and variations thereof. It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose systems that perform the specified functions or acts.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of this invention defined in the following claims.

We claim:

1. A cartridge for processing a sample, the cartridge comprising:

a first layer comprising a sample port and a fluid port at a broad surface of the first layer;

an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, and configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a void external to the waste chamber; and

a fluidic pathway, superior to the intermediate substrate and at least partially separated from the corrugated surface of the waste chamber by an elastomeric layer, wherein the fluidic pathway is fluidly coupled to the sample port and the fluid port, and wherein, upon deformation of the elastomeric layer at an occlusion position of the fluidic pathway, by way of the void of the corrugated surface, the fluidic pathway is configured to transfer waste fluid through an opening of the intermediate substrate and into an interior portion of the corrugated surface of the waste chamber.

2. The cartridge of claim 1, wherein the first layer is a unitary construction comprising a reagent port, the fluid port, and a detection chamber, and wherein the fluidic pathway is coupled to the reagent port, the fluid port, and the detection chamber.

3. The cartridge of claim 1, wherein the waste chamber comprises at least one waste inlet in communication with the opening of the intermediate substrate, and wherein the corrugated surface of the waste chamber further defines a rectangular prismatic void spanning a long dimension of the cartridge that defines a magnet housing region configured to reversibly receive a magnet from the direction perpendicular to a broad face of the first layer, and configured to cross the fluidic pathway.

4. The cartridge of claim 3, wherein the fluidic pathway comprises a capture segment, downstream of the sample port and configured to cross the magnet housing region multiple times.

5. The cartridge of claim 4, wherein the fluidic pathway is configured to transfer waste fluid of the sample to the waste chamber through a first waste inlet upstream of the capture segment by way of a first waste segment fluidly coupled to the first waste inlet and an initiating portion of the capture segment, and wherein the fluidic pathway is configured to transfer a second waste fluid to the waste chamber through a second waste inlet downstream of the capture segment by way of a second waste segment fluidly coupled to the second waste inlet and a terminating portion of the capture segment.

6. The cartridge of claim 1, wherein the elastomeric layer is situated between the fluidic pathway and the intermediate substrate, wherein the intermediate substrate provides access to a set of occlusion positions of the fluidic pathway through at least at a subset of a set of voids of the corrugated surface of the waste chamber, such that the fluidic pathway is configured to be occluded through the elastomeric layer at the set of occlusion positions from a direction perpendicular to the broad surface of the first layer.

7. The cartridge of claim 6, wherein at least one void of the set of voids defined by the corrugated surface of the waste chamber is defined by five surfaces of the corrugated surface, including a first pair of parallel surfaces and a second pair of parallel surfaces orthogonal to the first pair of parallel surfaces and orthogonal to the broad surface of the first layer, and a surface, coupled to the first pair of surfaces and the second pair of surfaces and proximal to the first layer, having a set of openings for occlusion of the fluidic pathway at the set of occlusion positions, and wherein occluding the fluidic pathway at a first subset of the set of occlusion positions defines a truncated pathway configured to facilitate transfer of waste fluid of the sample to the waste chamber.

8. A cartridge for processing a sample, the cartridge comprising:

a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and

a first fluidic pathway, formed by at least a portion of the first layer; and

a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate.

9. The cartridge of claim 8, wherein the elastomeric layer is inferior to the film layer.

10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection cham-

ber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.

11. The cartridge of claim 10, further comprising 1) a heating region defined as a recessed region of the first layer that is parallel to the set of voids of the corrugated surface, and 2) a vent region, such that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber.

12. The cartridge of claim 10, wherein the fluid port is fluidly coupled to the first fluidic pathway and to the second fluidic pathway.

13. The cartridge of claim 9, wherein the first detection chamber comprises a first serpentine-shaped fluidic channel, and the second detection chamber comprises a second serpentine-shaped fluidic channel.

14. The cartridge of claim 13, wherein the first serpentine-shaped fluidic channel comprises three wide channels directly interconnected by two narrow channels, wherein the three wide channels include a first wide channel comprising a detection chamber inlet into the first detection chamber and a second wide channel comprising a detection chamber outlet from the first detection chamber.

15. The cartridge of claim 8, wherein at least one void of the set of voids defined by the corrugated surface of the waste chamber is a rectangular prismatic void spanning a long dimension of the cartridge and defining a magnet housing region configured to cross the first fluidic pathway and the second fluidic pathway.

16. The cartridge of claim 8, wherein the elastomeric layer is situated between the first layer and the intermediate substrate, wherein the intermediate substrate provides access to a set of occlusion positions of the first fluidic pathway and of the second fluidic pathway by way of the set of voids of the corrugated surface of the waste chamber, such that the first fluidic pathway and the second fluidic pathway are configured to be occluded upon deformation of the elastomeric layer at subsets of the set of occlusion positions, and wherein the waste chamber is located inferior to the elastomeric layer.

17. The cartridge of claim 16, wherein the first fluidic pathway is configured to transfer a first waste fluid of the sample to the waste chamber through a first waste inlet, upon occlusion of a first subset of the set of occlusion positions, by way of a first waste segment fluidly coupled to the first waste inlet and a first portion of the first fluidic pathway proximal the first sample port, and wherein the first fluidic pathway is configured to transfer a second waste fluid of the sample to the waste chamber through a second waste inlet, upon occlusion of a second subset of the set of occlusion positions, by way of a second waste segment fluidly coupled to the second waste inlet and a second portion of the first fluidic pathway substantially downstream of the first sample port.

18. The cartridge of claim 16, wherein the first fluidic pathway is configured to transfer a first waste fluid to the waste chamber through a first waste inlet, upon occlusion of a first subset of the set of occlusion positions, by way of a first waste segment fluidly coupled to the first waste inlet and inline with the first fluidic pathway, and wherein the second fluidic pathway is configured to transfer a second waste fluid to the waste chamber through a second waste inlet, upon occlusion of a second subset of the set of occlusion positions, by way of a second waste segment fluidly coupled to the second waste inlet and inline with the second fluidic pathway.